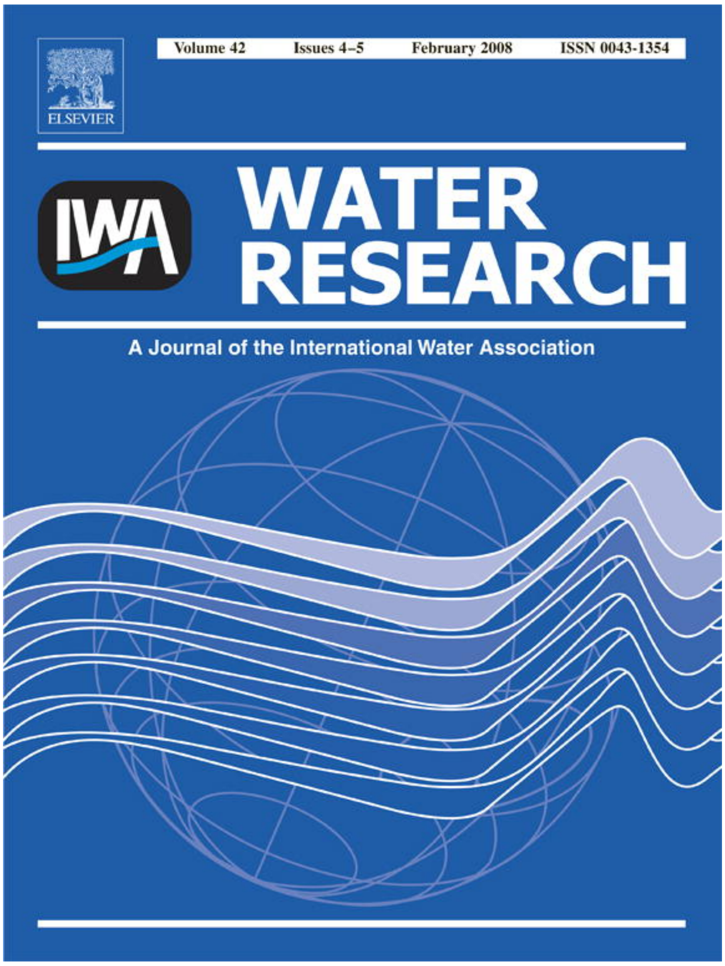
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Bioremediation efficiency in the removal of dissolved inorganic nutrients by the red seaweed, *Porphyra yezoensis*, cultivated in the open sea

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ABSTRACT

The bioremediation capability and efficiency of large-scale *Porphyra* cultivation in the removal of inorganic nitrogen and phosphorus from open sea area were studied. The study took place in 2002–2004, in a 300 ha nori farm along the Lusi coast, Qidong County, Jiangsu Province, China, where the valuable rhodophyte seaweed *Porphyra yezoensis* has been extensively cultivated. Nutrient concentrations were significantly reduced by the seaweed cultivation. During the non-cultivation period of *P. yezoensis*, the concentrations of NH_4-N, NO_2-N, NO_3-N and PO_4-P were 43–61, 1–3, 33–44 and 1–3 $\mu mol\,L^{-1}$, respectively. Within the *Porphyra* cultivation area, the average nutrient concentrations during the *Porphyra* cultivation season were 20.5, 1.1, 27.9 and 0.96 $\mu mol\,L^{-1}$ for NH_4-N, NO_2-N, NO_3-N and PO_4-P, respectively, significantly lower than in the non-cultivation season ($p<0.05$). Compared with the control area, *Porphyra* farming resulted in the reduction of NH_4-N, NO_2-N, NO_3-N and PO_4-P by 50–94%, 42–91%, 21–38% and 42–67%, respectively. Nitrogen and phosphorus contents in dry *Porphyra* thalli harvested from the Lusi coast averaged 6.3% and 1.0%, respectively. There were significant monthly variations in tissue nitrogen content ($p<0.05$) but not in tissue phosphorus content ($p>0.05$). The highest tissue nitrogen content, 7.65% in dry wt, was found in December and the lowest value, 4.85%, in dry wt, in April. The annual biomass production of *P. yezoensis* was about 800 kg dry wt ha^{-1} at the Lusi Coast in 2003–2004. An average of 14708.5 kg of tissue nitrogen and 2373.5 kg of tissue phosphorus in *P. yezoensis* biomass were harvested annually from 300 ha of cultivation from Lusi coastal water. These results indicated that *Porphyra* efficiently removed excess nutrient from nearshore eutrophic coastal areas. Therefore, large-scale cultivation of *P. yezoensis* could alleviate eutrophication in coastal waters economically.

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1. Introduction

Eutrophication is generally considered as the principal cause of the globally observed red tides and the deterioration of marine coastal environments (Schramm et al., 1996). Moreover, it has been accelerated by human activities in recent decades (Schramm, 1999; Capriulo et al., 2002). In China, coastal areas are the centers of population and industrialization. In recent years, fast growth in population and human activities, such as various agricultural practices, discharge of industrial wastewater, urban runoff, burning of fossil fuels and large-scale finfish and shrimp aquaculture, have resulted in the increase of nutrient inputs that are many times more than that generated by the natural processes (Victor et al., 2002). Today, coastal waters of China are the primary recipients of nutrients from land and finfish and/or shrimp aquaculture, and many areas exhibit typical symptoms of eutrophication (Xu and He, 2006). Rapid growth of intensive mariculture adds a continuous or pulsed release of nutrients, which contribute to coastal eutrophication (Troell et al., 2003; Neori et al., 2004). Increased nutrient inputs would generate an array of complex problems, including the excessive growth of harmful microalgae, which, in turn, may lead to more frequent occurrence of red tides (Fei, 2004).

Among different measures to control eutrophication, seaweed cultivation is receiving more and more attention, because of the low cost of cultivation and possible pollutant removal by the seaweed (Xu and He, 2006). The red seaweed *Porphyra* (Nori) is the most valuable maricultured seaweed in the world, worth approximately US$18,000/t dry biomass, with an annual market value of over US$ 1.3 billion (FAO, 2006). *Porphyra* grows fast and requires less than 40 days from seeding to the first harvest in a net culture. And it can be harvested repeatedly every 15 days (Sahoo and Yarish, 2005). Cultivated *Porphyra* grows well with higher nitrogen and other nutrients (Carmona et al., 2006). In *Porphyra yezoensis* cultivation, high yield and good quality were obtained in those regions where nitrogen concentrations (NO_3-N+NH_4-N) are over 100 mg m^{-3} ($>$7.2 mM) (S.E.P.E. and S.A.T.M., 1978; Fei, 2004). The high productivity, along with its higher capability (63–170% higher) to rapidly assimilate nitrogen and phosphorus nutrients than other seaweeds, makes *Porphyra* an excellent choice for eutrophication abatement via large-scale cultivation (Chopin and Yarish, 1998, 1999; Chopin et al., 1999; Neori et al., 2004). Moreover, *Porphyra* cultivation also provides a valuable product upon harvest (Cuomo et al., 1993).

Until now, however, most of the bioremediation studies have focused on the green seaweeds *Ulva*, the red seaweed *Gracilaria* (Anderson et al., 1999; Mariachiara and Pierluigi, 2002; Suzuki et al., 2005; Hernández et al., 2006; Yang et al., 2006) and the kelp, *Laminaria* (Chopin et al., 2003; Yang et al., 2004) for nutrient bioremediation. The cultivations of *Ulva* and *Gracilaria* are well established, but their market value is lower than that of *Porphyra*. It is highly desirable to develop the cultivation of the high-valued seaweed species, such as *Porphyra*, to reduce eutrophication and improve the quality of coastal waters.

Previous investigations have been carried out using cultured gametophytes of *Porphyra* in the laboratory as potential biofilters. They include *P. amplissima*, *P. leucosticta*, *P. purpurea* and *P. umbilicalis* from the east coast of North America; *P. haitanensis*, *P. katadai*, *P. yezoensis* and *P. dentata* in Asia; and *P. dioca* in Europe (Chung et al., 2002; Kraemer et al., 2004; Carmona et al., 2006; Pereira et al., 2006). The positive role of *Porphyra* in removing nitrogen and phosphorus at the sites of experimental nori/salmon integrated aquaculture has also been reported (Chopin and Yarish, 1999; Chopin et al., 1999; McVey et al., 2002). However, to our knowledge, there are few published studies in which cultivated *Porphyra* is used to remediate coastal water eutrophication in an open-water system, where changes in the water environment and nutrients are difficult to monitor and control.

The aim of the present study was to investigate the fluctuation of the levels of dissolved inorganic nutrients in seawater along the Lusi coast of China and the bioremediation potential of large-scale cultivation of *Porphyra yezoensis* to alleviate coastal eutrophication. Simultaneously, the biomass production and seasonal variations of the levels of tissue nitrogen and phosphorus of *P. yezoensis* are reported during this 20-month assessment.

2. Materials and methods

2.1. Study location

The study was conducted at the Lusi nori farm (121°35′ E, 32°05′ N), with an area of 300 ha of semi-floating raft cultivated *Porphyra yezoensis* (Sahoo and Yarish, 2005) at about 1 km distance away from the shore. Lusi Port, a small town situated north of Qidong City in Jiangsu Province of China, sits on the northern bank of the mouth of the Yangtze River and has a long coastline that runs for more than 40 km (Fig. 1). It is one of China's four richest areas in fishery resources. The

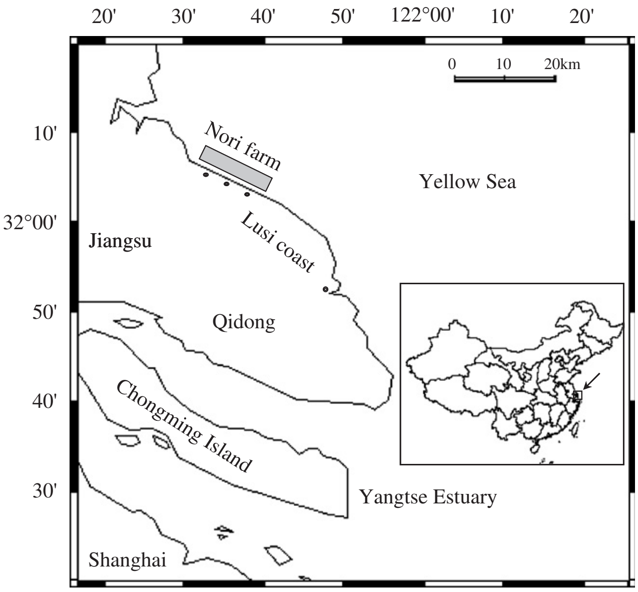

Fig. 1 – Map of the Lusi coast, Qidong County, Jiangsu Province in China, showing the location of the *Porphyra yezoensis* cultivation.

hydrography and dynamics of the Lusi coast have been described by Chen and Shen (1999). The experimental work was carried out from October 2002 to May 2004. The *P. yezoensis* cultivation season occurs from September to April every year. Twelve sampling sites were set at four locations (Dayang, Haifeng, Maojia and Tanglu Harbors) with three stations, at Dayang, Haifeng and Maojia (Fig. 2). There was no *P. yezoensis* cultivation in Tanglu Harbor, which served as a control site for water quality measurements.

2.2. *Sampling*

2.2.1. *Water samples*

Water samples were collected once a month and each sampling occurred during the time of slack tide. On each sampling site, three 250 ml water samples ($n = 3$) for dissolved nutrient analysis were taken from 15 to 20 cm and 100 to 105 cm below the surface. In situ temperature, pH, salinity and dissolved oxygen were measured using a HANNA meter at the two depths. Chemical oxygen demand (COD), biochemical oxygen demand (BOD) and chlorophyll *a* were monitored by an AWA analyzer. The water samples were filtered through cellulose membrane filters (Millipore® HAWP 0.45 μm pore) and kept on ice (about 4 °C) until measurement in the laboratory. For the measurement of ammonia nitrogen (NH_4-N), the indophenol blue method as described by Grasshoff et al. (1983) was used. For the measurement of nitrate nitrogen (NO_3-N), the cadmium–copper reduction method as described by Wood et al. (1989) was used, and for the measurement of nitrite nitrogen (NO_2-N), the Griess-Ilosvay method as described by Benschneider and Robinson (1952) was followed. For the measurement of phosphate phosphorus (PO_4-P), the phosphomolybdenum blue method as described by Murphy and Riley (1962) was followed. Each sample was sub-sampled and analyzed in triplicate. The results reported represented the mean values of three independent samples collected in the field for each sampling date.

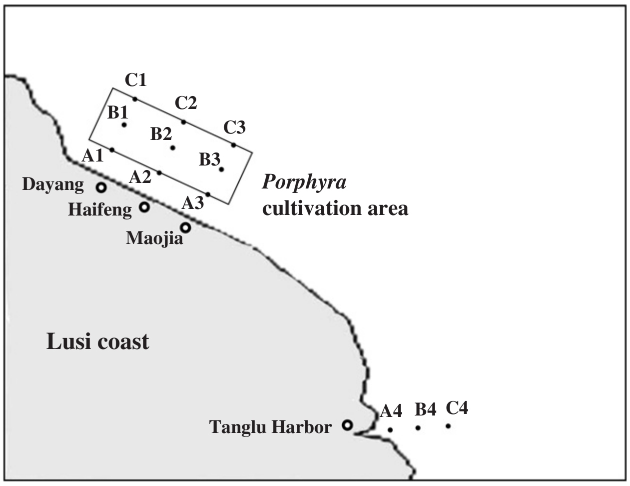

Fig. 2 – Schematic map of the Lusi coast, showing the locations of the 12 sampling sites. *Porphyra yezoensis* cultivation farm situates on the sea surface of Dayang Harbor, Haifeng and Maojia Harbors. There is about 10 km between Dayang Harbor and Maojia Harbor, and Haifeng is in the middle. Tanglu Harbor at the southern end of the Lusi coast is about 40 km away from Maojia Harbor. Sampling site A1, B1 and C1 were set at about 1, 2 and 3 km, respectively, from shore at Dayang Harbor. Sampling site A2, B2 and C2 at Haifeng; A3, B3 and C3 at Maojia Harbor; A4, B4 and C4 at Tanglu Harbor, were also similarly set. A1, A2 and A3 situated on the inshore side of the *P. yezoensis* cultivation area; B1, B2 and B3 located at the center of the *P. yezoensis* cultivation area; and C1, C2 and C3 located on the offshore side of the cultivation area.

2.2.2. *Seaweed samples*

Blades of *P. yezoensis* cultivated on semi-floating rafts along the Lusi coast were collected in the early morning and washed with filtered seawater (by 0.45 μm filter membrane) to remove epiphytes, sediment and detritus. The blades were placed in sealed plastic bags and kept on ice until preparation in the laboratory. In the laboratory, samples were then gently brushed under running filtered seawater, briefly rinsed with distilled water three times and dried at 60 °C for 3 days. The dried material was ground to powder and kept in desiccators containing silica gel at room temperature until chemical analysis.

2.3. *Tissue analysis*

Total nitrogen and phosphorus contents in *P. yezoensis* blade tissue were determined after peroxymonosulphuric acid digestion (Hach et al., 1987) in triplicates for each sample. Specifically, samples containing 100 mg (dry matter) were digested with 4 ml concentrated sulfuric acid at 440 °C and treated with 17 ml of 30% hydrogen peroxide. Total nitrogen and phosphorus contents in the samples were determined spectrophotometrically after chemical reactions (Lourenço et al., 2005). An estimate of total nitrogen and phosphorus removal by *P. yezoensis* (N, P yield) was made by multiplying *P. yezoensis* biomass yield during two cultivation seasons with nitrogen and phosphorus contents of the seaweed tissue (Schuenhoff et al., 2006).

2.4. *Statistical analysis*

Statistical analyses followed the methods outlined by Zar (1996). Data were tested for homogeneity of variance using Bartlett's test and then submitted to a one-way ANOVA with the Statistics 6.0 of Microsoft Windows. If *F* values showed significance, individual means were compared using Tukey's honest significant difference. Differences were considered significant when $p < 0.05$.

3. Results

3.1. *Eutrophication along the Lusi coast*

The changes in the water temperature of the Lusi coast during the monitoring period followed the typical seasonal pattern of the Yellow Sea. Summer values varied between

23.20 and 30.98 °C, while during the winter the temperature ranged from 6.26 to 13.74 °C. The annual mean pH was 8.05 and the highest values (8.30) were recorded during the cold periods. Salinity in the water column ranged between 28.37 and 32.75 psu. Along the Lusi coast, *P. yezoensis* cultivation begins on about September 20th to the middle of April of the following year. From May to August, there is no *P. yezoensis* cultivation. The seawater conditions during this period indicate eutrophic conditions. Table 1 shows the dissolved nutrient, DO, BOD, COD and chlorophyll *a* concentrations during the non-cultivation period at Dayang, Haifeng, Maojia Harbors and at Tanglu Harbor. The concentration ranges of NH_4-N, NO_2-N, NO_3-N and PO_4-P were 43.0–61.4, 1.1–2.8, 33.3–43.8 and 1.2–2.8 $\mu mol\,L^{-1}$, respectively. Ammonia and nitrate were the dominant forms of nitrogen, which accounted for 54.7–55.4% and 39.5–42.4% respectively. And the DO, BOD, COD and chlorophyll *a* concentrations were in the ranges of 4.0–4.4, 3.0–3.5, 3.4–4.2 $mg\,L^{-1}$ and 6.6–12.7 $\mu g\,L^{-1}$, respectively. High levels of expression of eutrophic conditions occurred along all sites near the Lusi coast.

3.2. *Variation of the concentrations of the dissolved nutrients in Lusi coastal waters*

The monthly changes of nitrogen and phosphorus concentrations in the water column of the Lusi coast are shown in Fig. 3a–d. Turbid waters prevailed aperiodically along the Lusi coast in 2002–2004. The maximum nutrient concentrations were observed at the end of 2002. As a result of measures by the Chinese Environmental Protection Agency to reduce nutrient discharges from untreated or partially treated sewage effluent from surrounding factories, nutrients levels had been in decline since January, 2003.

From October 2002 to May 2004, data of dissolved nutrient concentrations in the non-cultivation area of the Lusi coast (Tanglu Harbor) indicated that coastal ammonium concentrations were generally less than 70 $\mu mol\,L^{-1}$. An exception occurred in December 2002, when there was a sharp increase of ammonium loading in these waters (a maximum of 171.3 $\mu mol\,L^{-1}$; c.f. Fig. 3a). Monthly values in nitrite and phosphorus concentrations showed a similar pattern as ammonia. The highest concentrations reached 6.3 for nitrite and 3.3 $\mu mol\,L^{-1}$ for phosphorus at Tanglu Harbor in December 2002 (Fig. 3b, d). Nitrite showed the lowest concentrations among dissolved inorganic nitrogen sources, typically lower than 3.0 $\mu mol\,L^{-1}$. NO_3-N showed higher concentrations than NO_2-N at the non-cultivation area, varying between 30.0 and 48.2 $\mu mol\,L^{-1}$ in most cases (Fig. 3c). The low levels of phosphate at different sites fluctuated between 1.5 and 3.3 $\mu mol\,L^{-1}$.

In the coastal waters of Tanglu Harbor (non-cultivation area), there were no significant differences in the average nutrient concentrations between the *Porphyra* cultivation season and the non-cultivation season ($p > 0.05$). However, the average nutrient concentrations in the *Porphyra* cultivation area (Dayang, Haifeng and Maojia Harbors) were significantly lower than in the non-cultivation area (Tanglu Harbor). This was especially pronounced during the *Porphyra* cultivation season ($P < 0.01$). From September 2003 to April 2004, NH_4-N concentration in Lusi coastal waters decreased

Table 1 – Concentrations of nitrogen and phosphorus nutrients, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and chlorophyll *a* along Lusi coast during *P. yezoensis* non-cultivation period

Stations	Dayang Harbor			Haifeng			Maojia Harbor			Tanglu Harbor		
(concentrations)	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
NH_4-N ($\mu mol\,L^{-1}$)	57.39±5.00	42.97±3.61	54.96±3.64	58.53±7.14	46.47±5.71	55.39±6.86	61.38±2.64	54.96±3.57	56.39±2.71	58.42±2.14	48.21±3.01	55.68±2.13
NO_2-N ($\mu mol\,L^{-1}$)	2.84±0.28	1.11±0.14	2.06±0.21	2.62±0.36	1.17±0.15	2.03±0.22	2.27±0.38	1.20±0.14	1.78±0.29	2.73±0.11	1.15±0.13	1.98±0.30
NO_3-N ($\mu mol\,L^{-1}$)	42.83±1.43	33.26±2.14	39.26±1.85	42.83±0.71	38.22±2.57	39.97±1.56	39.97±2.86	38.54±2.86	38.26±2.85	43.81±2.49	36.46±2.15	39.45±2.48
PO_4-P ($\mu mol\,L^{-1}$)	2.64±0.21	1.23±0.14	2.35±0.11	2.78±0.36	1.63±0.42	2.34±0.38	2.50±0.28	2.14±0.16	2.42±0.22	2.69±0.32	1.85±0.15	2.37±0.24
DO ($mg\,L^{-1}$)	4.22±0.11	4.01±0.09	4.12±0.20	4.19±0.05	4.05±0.05	4.13±0.07	4.40±0.18	3.98±0.14	4.18±0.03	4.34±0.30	4.02±0.35	4.15±0.13
BOD ($mg\,L^{-1}$)	3.27±0.05	2.99±0.08	3.11±0.02	3.42±0.23	3.02±0.09	3.15±0.25	3.29±0.38	3.13±0.42	3.21±0.15	3.48±0.03	3.01±0.18	3.16±0.22
COD ($mg\,L^{-1}$)	4.22±0.12	3.55±0.09	3.82±0.03	4.23±0.14	3.42±0.18	3.95±0.11	4.08±0.32	3.63±0.28	3.85±0.23	4.16±0.14	3.57±0.07	3.87±0.15
Chlorophyll *a* ($\mu g\,L^{-1}$)	9.82±1.18	7.34±0.29	9.76±0.60	9.99±0.75	7.66±1.29	9.87±0.65	12.71±0.26	6.63±0.28	9.92±0.53	10.05±1.86	7.44±0.78	9.98±0.45

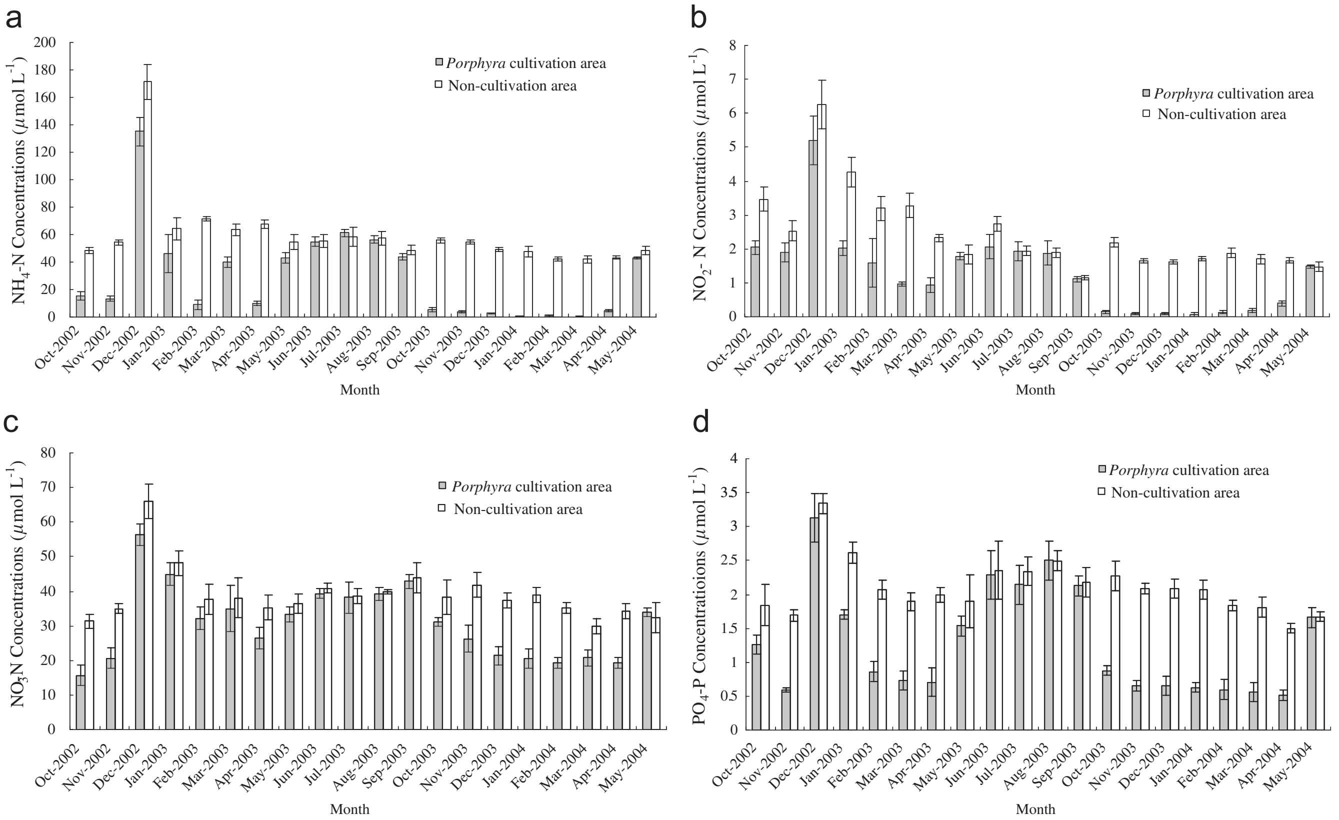

Fig. 3 – Monthly average concentrations of dissolved inorganic nitrogen (NH_4–N, NO_2-N, NO_3-N) and phosphate from October 2002 to May 2004 in Lusi coastal waters of the *Porphyra* cultivation area (Dayang Harbor, Haifeng and Maojia Harbors; $n = 27$; full bars) and the non-cultivation area (Tanglu Harbor; $n = 9$; open bars). Values are the mean ± SD.

Table 2 – Effects of *P. yezoensis* cultivation on dissolved nutrient concentrations ($\mu mol\,L^{-1}$) in Lusi coastal waters

Experimental areas	NH_4-N	NO_2-N	NO_3-N	DIN	PO_4-P
Oct 2002– Apr 2003					
Porphyra cultivation area ($n = 27$)	38.38 ± 3.84	2.09 ± 0.34	33.03 ± 2.46	73.51 ± 3.78	1.28 ± 0.14
Non-cultivation area ($n = 9$)	77.29 ± 5.36	3.62 ± 0.48	41.65 ± 2.63	122.56 ± 9.05	2.21 ± 0.32
Percentage of reduction (%)	50.34 ± 4.35	42.26 ± 1.37	20.70 ± 2.25	40.02 ± 5.26	42.08 ± 3.94
Oct 2003– Apr 2004					
Porphyra cultivation area ($n = 27$)	2.69 ± 0.50	0.16 ± 0.02	22.71 ± 3.60	25.55 ± 3.59	0.64 ± 0.12
Non-cultivation area ($n = 9$)	47.83 ± 2.33	1.77 ± 0.08	36.56 ± 2.68	86.16 ± 3.38	1.95 ± 0.23
Percentage of reduction (%)	94.38 ± 5.91	90.96 ± 8.45	37.88 ± 2.93	70.34 ± 5.87	67.18 ± 4.90

from 43.98 (±2.46) to 4.42 (±0.82). Nitrite-nitrogen concentrations were reduced from 1.12 (±0.08) $\mu mol\,L^{-1}$ to 0.40 (±0.07) $\mu mol\,L^{-1}$, and the NO_3-N concentration dropped from 42.83 (±2.00) to 19.40 (±1.57) $\mu mol\,L^{-1}$. Finally, the PO_4-P concentration decreased from 2.13 (±0.14) to 0.51 (±0.07) $\mu mol\,L^{-1}$. After the *Porphyra* harvest season in April 2004, higher nutrient contents in the water column were detected again in May 2004.

3.3. *Nutrient removal efficiency*

Table 2 shows the effects of *Porphyra yezoensis* cultivation on the concentrations of the dissolved nutrients in Lusi coastal waters. From October 2002 to April 2003, concentrations of dissolved inorganic nitrogen (DIN) exceeded 73 $\mu mol\,L^{-1}$, reflecting the added nitrogen inputs. And NH_4-N was the main form of inorganic nitrogen accounting for 52.21% of DIN in the cultivation area. Decreases of inorganic nitrogen and phosphorus concentrations in the seawater were detected after the next *Porphyra* cultivation season, from October 2003 to April 2004 ($p < 0.05$). Average DIN concentration in the cultivation area decreased to 25.55 (±3.59) $\mu mol\,L^{-1}$. The largest reduction of dissolved inorganic nitrogen was observed in the NH_4-N (94.38%), and the reduction of NO_2-N (90.96%) was significantly higher than that of NO_3-N (37.88%) during the experimental period of *P. yezoensis* cultivation ($p < 0.05$).

3.4. *Variation of tissue nitrogen and phosphorus contents*

Monthly changes in the average tissue nitrogen and phosphorus contents between December 2002 and April 2004 are shown in Fig. 4. Tissue nitrogen contents exhibited wider variations during the five month of cultivation than tissue phosphorus content. There was a marked decrease in the tissue nitrogen content of *Porphyra* ($p<0.05$), with losses as high as 36.6%. At the beginning of the harvest period, tissue nitrogen content reached the highest value of 7.65 (±0.81)% dry weight (DW). The lowest value was 4.85 (±0.62)% DW, which occurred at the end of the harvest period. There was no statistical difference in the tissue phosphorus of the *Porphyra* thalli ($p>0.05$) among the harvest months. Contents of phosphorus ranged from 0.88 (±0.18) % DW to 1.07 (±0.15) % DW from December to April.

3.5. *Production and bioremediation capability of large-scale Porphyra aquaculture*

Changes in monthly biomass production and total tissue nitrogen and phosphorus contents are shown in Fig. 5. Although there were variations in monthly production ($p<0.05$), after 7 months of cultivation, *P. yezoensis* showed consistent biomass yields with 241.4 t dry wt in 2002–2003 and 240.4 t DW in 2003–2004. Growth was the highest in late winter and early spring (January–March), with the highest production exceeding 250 kg ha^{-1}. Production declined during April. According to the levels of nitrogen and phosphorus content at harvest for the cultivation area of 300 ha, 15133.2 kg tissue nitrogen and 2413.2 kg tissue phosphorus in 2002–2003 and 14283.8 kg tissue nitrogen and 2333.8 kg tissue phosphorus in 2003–2004 were removed by harvesting *Porphyra*.

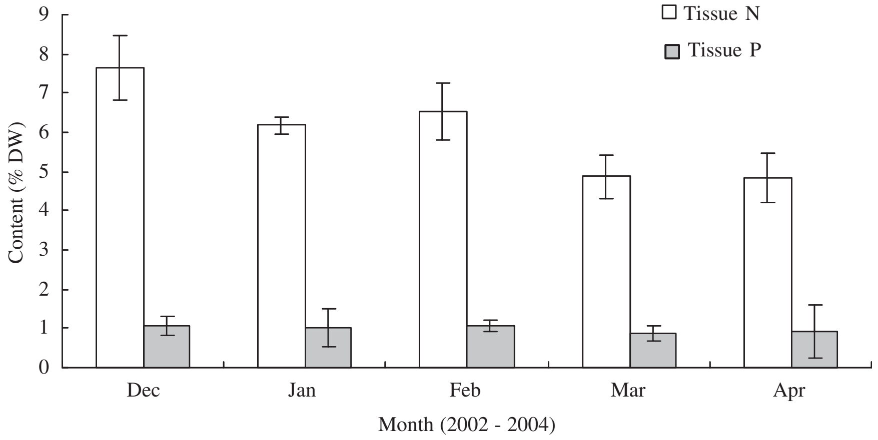

Fig. 4 – Mean values of nitrogen (open bars) and phosphorus (full bars) in the tissues of *Porphyra yezoensis* collected at the Lusi nori farm. Data are expressed as percentage of the dry weight (DW) and each bar represents the mean of four to six replicates ± SD.

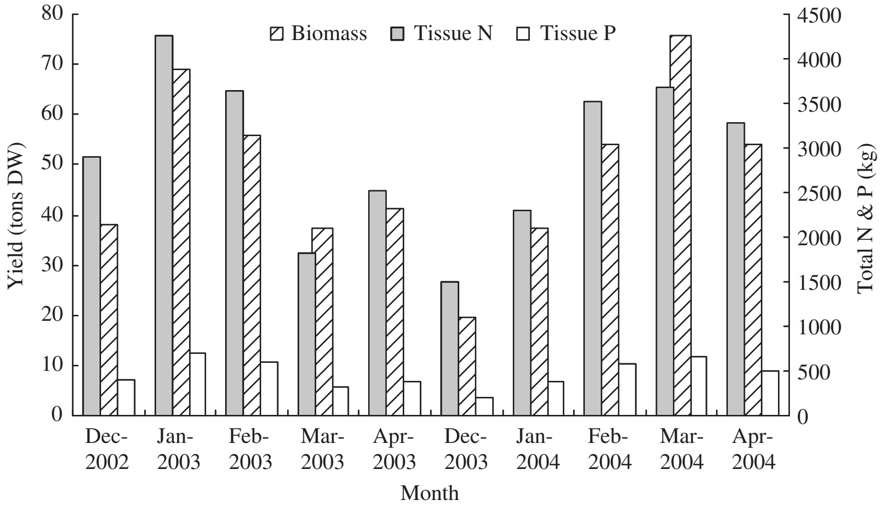

Fig. 5 – Monthly biomass production (line bars) and total tissue nitrogen (full bars) and phosphorus (open bars) contents of harvested *Porphyra* of the Lusi nori farm in 2002–2004.

4. Discussion

4.1. *Eutrophication along the Lusi coast*

According to the Chinese National Standards (S.E.P.A.C, 1998), the eutrophication along the Lusi coast was severe, with concentrations of total dissolved inorganic nitrogen and phosphorus reaching 77.4–108.0 and 1.2–2.8 μmol L^{-1}, respectively, during the period of no cultivation of *Porphyra*. The severe eutrophic conditions occurring along the Lusi coast is similar to those of other open coastal systems along the Chinese coast, such as Xiangshan Harbor and Yaling Bay. In theses areas, the DIN and DIP concentrations in the water column are 57.1–85.6 and 1.1–2.6 μmol L^{-1}, respectively (Ye et al., 2002; Jia et al., 2005). In the months with normal precipitation, the total nutrient loadings of the coastal waters around Lusi were dominated by point-source discharges, including contributions from 32 fish farms as well as municipal and industrial effluents (Zhang et al., 2005). In the winter of 2002, the discharge of large volumes of sewage from cold storage factories in the area had brought a series of severe ammonium effluent releases. These effluent releases caused massive deaths of shellfish, which were washed up on the beaches of Lusi, resulting in economic losses of at least US $630,000 (E.P.B.J.P, 2004). This event led to the implementation of an Aquatic Environment Plan by the local government. By the spring of 2003, strict implementation of the Plan brought reductions in point-source discharges of total nitrogen and phosphorus from land by 52.1% and 21.6%, respectively, compared with the levels 3 months earlier. The overall ratios of nitrogen and phosphorus indicated that phosphorus was by far the limiting nutrient in the coastal waters of Lusi. Even the lowest N:P ratio (37.4:1) surpassed the threshold at which phosphorus would become limiting (about 25:1) (Guildford and Hecky, 2000). These results indicated that any efforts to reduce eutrophication in the coastal waters of Lusi must focus on nitrogen removal.

4.2. *The role of porphyra aquaculture in reducing coastal eutrophication*

Substantial reductions in point-source discharges decreased the nutrient levels along the Lusi Coast (E.P.B.J.P, 2004). Our results indicated that cultivation of *Porphyra* further reduced the levels of dissolved inorganic nitrogen in the coastal water of Lusi during the study period (2002–2004). Average monthly DIN concentrations decreased from 86.16–122.56 μmol L^{-1} in non-cultivation areas to 25.55–73.51 μmol L^{-1} in *Porphyra* cultivation areas. At the same time, the DIP concentrations were reduced from 1.95–2.21 μmol L^{-1} in the non cultivation areas and to 0.64–1.28 μmol L^{-1} in the cultivation areas. In this study, the annual mean reduction for DIN was 55.2% in *Porphyra* cultivation areas. The annual mean reduction for DIP was 54.6%. The nutrient removal efficiencies obtained in this study for *Porphyra* were much greater than those in *Gracilaria lemaneaformis* cultivation areas of the eutrophic coastal waters near Dongshan Island in Fujian Province, China (Tang et al., 2005). At Dongshan Island, the removal efficiencies of DIN and DIP by *G. lemaneaformis* mariculture were less than 12% and 25%, respectively. In one cultivation season, the harvest of *P. yezoensis* had removed 49 kg ha^{-1} of tissue nitrogen and 7.9 kg ha^{-1} of tissue phosphorus from the coastal waters of Lusi. Troell et al. (1997) reported that the harvest of *Gracilaria chilensis* near open fish cages had the potential to remove 6.5% of the dissolved inorganic nitrogen effluents as well as 27% of dissolved phosphorous effluents from seawater. Therefore, *P. yezoensis* should be considered a good bioremediation candidate in reducing marine eutrophication as it is very favorable compared with other species of seaweed (Fei, 2004; Kraemer et al., 2004).

Under laboratory conditions, Carmona et al. (2006) reported that *P. yezoensis* showed nutrient removal capability of 92–93% for nitrogen and 72–85% for phosphorus in short periods of experiments for 3–4 days. However, in open-water systems, there are few published studies on the bioremediation of eutrophic coastal waters by cultivated *Porphyra* (Fei, 2004). Nutrient uptake efficiencies by seaweeds are relatively low in open-water systems due to the 3-D hydrographic nature of the water flow (Troell et al., 1997, 2003). Further studies on the open-water bioremediation potential of seaweeds in dynamic open-water environments is still needed (Troell et al., 1997, Chopin et al., 1999; Neori et al., 2004).

4.3. *Effects of nitrogen source variations on nitrogen uptake and growth of Porphyra*

Nutrient concentrations and the form of the limiting nutrient (NH_4-N vs. NO_3-N) may influence uptake rates and growth of seaweed (Harrison and Hurd, 2001). In the ocean-based farm, greater reduction of NH_4-N (50–94%) than NO_3-N (21–38%) by *P. yezoensis* was observed. An annual yield of 802.95 ± 1.75 kg dry wt ha^{-1} was obtained when NH_4-N was the principal nutrient in the water. In laboratory experiments, Wu et al. (1984) also reported that their strain of *P. yezoensis* had a preference for NH_4-N rather than NO_3-N. Higher uptake rates of NH_4-N were also observed in *Gracilaria tenuistipitata* (Haglund and Pedersén, 1993) and in *U. lactuca* (Neori et al., 1996). Many other seaweeds take up NH_4-N preferentially over NO_3-N and therefore NH_4-N inhibits the uptake of NO_3-N by up to 50% (DeBoer, 1981). However, Carmona et al. (2006) and Hanisak (1990) showed that *Porphyra* species and *Gracilaria tikvahiae* had similar growth rates irrespective of the nitrogen sources. In contrast, Hafting (1999) reported that under high light conditions (160 μmol m^{-2} s^{-1}) NO_3-N was a better nitrogen source for the growth of *P. yezoensis* than NH_4-N. Under low light conditions (50 μmol m^{-2} s^{-1}), there was no difference in the growth rates with NO_3-N or NH_4-N. And Shen et al. (2006) showed that NO_3-N was better than NH_4-N as the nitrogen source for vegetative cells of *P. yezoensis* cultured on solidified agar medium.

4.4. *Tissue nutrient contents and nutrient storage*

The comparison of water-column inorganic N:P ratios to algal tissue N:P ratios indicated that the productivity of *P. yezoensis* along the Lusi coast is limited by phosphorus instead of nitrogen (Harrison and Hurd, 2001). The average N:P molar ratio in *P. yezoensis* is 13.31 ± 0.27 and a molar N:P ratio of 13–15 is optimal for growth (Hafting 1999). In eutrophic systems, the amount of nutrients stored as seaweed biomass is important at the ecosystem level (Valiela et al., 1997). The average internal tissue nitrogen level was 6.3% (±1.4%) DW and tissue phosphorus content was 1.0% (±0.2%) DW in this study. Our results are similar to the studies of Chopin et al. (1999), where tissue concentrations of nitrogen exceeded 6% near salmon net pens. The ability of *P. yezoensis* to store large amounts of nitrogen and phosphorus in their tissue may provide an efficient way to remove nutrients from eutrophic seawater. Some nitrogen, not used immediately for growth, can be sequestered as photosynthetic pigments, free amino acids and proteins (Martinez and Rico, 2002). Phosphorus can be stored in algae in the vacuoles, as phosphorylated metabolites, in polyphosphate vesicles, or as polyphosphate granules in the cytoplasm and macroscopic gametophytes (Chopin et al., 2004). Thus, it is believed that cultivated *P. yezoensis* have actually played the role of cleaning their surrounding environment by storing large quantities of nutrients.

5. Conclusions

1. Results from this study suggested that the eutrophication along the Lusi coast was severe when *Porphyra* was not cultivated. Increased nutrient inputs promoted a complex array of symptoms, including high levels of chlorophyll *a*, reduced concentrations of DO and increased COD and BOD in Lusi coastal waters.
2. In the open-water system, the cultivation of *P. yezoensis* on a large-scale efficiently removed the dissolved nutrients and improved water quality in the eutrophic coastal area. *Porphyra* farming resulted in the reduction of NH_4-N, NO_2-N, NO_3-N and PO_4-P by 50–94%, 42–91%, 21–38% and 42–67%, respectively. Moreover, reductions of NH_4-N and NO_2-N were more than that of NO_3-N.
3. Based on the average internal tissue nitrogen (6.3% dry wt) and tissue phosphorus (1.0% dry wt) level, as well as the cultivation productivity (about 800 kg dry wt ha^{-1}) of *P. yezoensis* at the Lusi coast in 2003–2004, an average of 14708.5 kg of nitrogen and 2373.5 kg of phosphorus in *P. yezoensis* biomass from 300 ha were removed annually from eutrophic coastal water. Our research supported the feasibility of developing promising *P. yezoensis* aquaculture for eutrophication abatement in coastal waters.

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