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Biofiltration efficiency and biochemical composition of three seaweed species cultivated in a fish-seaweed integrated culture

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We evaluated the potential of three seaweed species (*Ulva pertusa*, *Saccharina japonica*, *Gracilariopsis chorda*) as biofilters for effluents from black rockfish (*Sebastes schlegeli*) tanks. The experiments consisted of a fish monoculture system and a fish-seaweed integrated system under identical physical conditions. All species efficiently removed NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-} from the fish tank effluents. Of the three species evaluated, *U. pertusa* showed the highest biofiltering efficiency for NH_4^+ (>80%). In contrast to *U. pertusa* and *G. chorda*, *S. japonica* showed a relatively higher preference for $NO_3^- + NO_2^-$ than for NH_4^+. These results suggest that seaweeds may select nitrogen sources fitting their storage capacity. Therefore, standard fish farm effluents should establish a total nitrogen concentration that includes both NO_3^- and NH_4^+, and the selection of a biofilter seaweed species should be made with consideration of the N forms expelled in effluent. The biofiltering efficiency for PO_4^{3-} was highest in *G. chorda* (38.1%) and lowest in *S. japonica* (20.2%). In all species, tissue N and P contents rapidly increased over the initial values. The data for tissue N and P contents, and C : N and N : P ratios, indicate that neither N nor P was limiting. This suggests that the three species serve as biofilters by storing large amounts of nutrients. These results provide valuable information for selecting optimal seaweed species in fish-seaweed integrated systems and allow land-based integrated aquaculture system operators to understand the behavior of integrated cultures sufficiently for the results herein to be extrapolated to larger-scale cultures.

Key Words: biofiltration; land-based aquaculture system; nitrogen; seaweed; tissue nutrient

INTRODUCTION

Annual consumption of seafood has increased dramatically over the past three decades worldwide, but supply from wild capture fisheries appears to have reached an uppermost limit (Food and Agriculture Organization of the United Nations 2007). Accordingly, aquaculture production has been growing by more than 10% annually and will reach 50% of world's seafood supply in 2030 (Food and Agriculture Organization of the United Nations 2007). However, intensive fish aquaculture has caused serious ecological problems, such as coastal eutrophication due to the release of excess nutrients (Read and Fernandes 2003). Moreover, this release may negatively influence the aquaculture activity itself by increasing the ammonium toxicity and water turbidity (Troell et al. 1999). Accordingly, various approaches are being taken by government authorities to reduce excess nutrients in effluents, including effluent regulations that limit maximum allowed nutrient concentrations in effluents

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discharged by aquaculture facilities (Tacon and Forster 2003). Ryther et al. (1975) suggested a biofilter system based on the seaweed nutrient uptake rate for reducing nutrients in effluents. Specifically, they developed an integrated multi-trophic aquaculture (IMTA) system, which combined the cultivation of fed aquaculture species (e.g., finfish / shrimp) with that of organic extractive aquaculture species (e.g., shellfish) and inorganic extractive aquaculture species (e.g., seaweed) for balanced ecosystem management (Chopin and Robinson 2004).

Previous studies have demonstrated that seaweed-based integrated aquaculture systems are able to improve water quality and environmental performance by removing nutrients, and that they have the potential to yield additional profits from seaweed production (Buschmann et al. 1996, Phang et al. 1996). Integrating optimal seaweed species into an aquaculture system is very important to provide sustainable environmentally friendly aquaculture. The selection of seaweed species for use in an integrated aquaculture system must involve consideration of both their economic value (e.g., marketable species) and their biofiltration capacity (e.g., nutrient uptake rate, growth rate, and tissue nitrogen concentration) (Neori et al. 2004). Moreover, the choice of seaweed depends on a match between the ecophysiological characteristics of the species and the environmental conditions present in the farm (Kang et al. 2008).

Nitrogen plays an important role in controlling algal growth in marine environments, and nitrogen uptake rates by macroalgae depend on the concentrations of nitrogen sources (Lobban and Harrison 1994). Hanisak (1990) reported that the preference for nitrogen sources is influenced by the nitrogen status of the seaweed. Wu et al. (1984) demonstrated that NH_4^+ is a better N source for *Porphyra yezoensis* than NO_3^-, while *Nereocystis* showed a significant preference for NO_3^- under high concentrations (Ahn et al. 1998). The growth of *Gracilaria cornea* presented similar rates, regardless of whether NH_4^+ or NO_3^- was provided (Navarro-Angulo and Robledo 1999). Therefore, the form of nitrogen produced can be an important factor when selecting seaweed species for application in an integrated aquaculture system since the effluents discharged from fish aquaculture usually contain NO_3^- and NO_2^- in addition to NH_4^+.

Excessive nitrogen in effluent streams induces changes in the biochemical composition of macroalgae (Lahaye et al. 1995). For example, *Porphyra* species exhibit higher tissue nitrogen concentrations under high nitrogen levels (Korbee et al. 2005, Kang et al. 2009). Jones et al. (2002) reported that the responses in seaweed growth and pigment concentrations were correlated with responses in tissue nitrogen (N) content. Moreover, tissue N content in seaweed exposed to high nitrogen concentrations in integrated seaweed-fish aquaculture was markedly increased when compared with that of the initial thalli (Zhou et al. 2006). Occasionally, seaweed incubated under phosphate deficiency shows a decrease in tissue P content, which may negatively affect nutrient removal efficiency or growth (Martínez-Aragón et al. 2002). Furthermore, the nutrient removal efficiency of seaweed is affected by other factors, including tissue C : N and N : P ratios (Chopin et al. 2001).

The purpose of the present study was to evaluate the capabilities of *Ulva pertusa*, *Saccharina japonica*, and *Gracilariopsis chorda* to biofilter enriched nutrients from fish effluents in land-based integrated aquaculture systems. The three species were selected because they have high marketability (*G. chorda* and *S. japonica*) or are already known to provide efficient biofiltration (*U. pertusa*) (Hernández et al. 2006). In addition, we investigated nitrogen form-specific reduction efficiency in these three species to determine the appropriate seaweed species for each nitrogen source found in the effluents. Moreover, we measured the tissue nutrient contents in each species before and after the experiment.

MATERIALS AND METHODS

Plant materials

The seaweeds used in the experiments were collected in two bays from June to August 2007 and were transported to the aquaculture research center at Chonnam National University (34°37' N, 127°43' E) in Dolsando, Korea. The green seaweed, *U. pertusa*, were collected from a rocky shore in Gamak Bay (34°35' N, 127°43' E), while the brown seaweed, *S. japonica*, and the red seaweed, *G. chorda*, were collected from a seaweed farm located in Deukryang Bay (34°37' N, 127°14' E). The samples were rinsed with filtered seawater and gently scrubbed to remove sediments and epiphytes. The specimens were then pre-cultured for 3-4 days under a continuous flow of filtered seawater pumped from Gamak Bay (mean nutrient concentrations were 0.6 μM NH_4^+, 0.5 μM $NO_3^- + NO_2^-$, and 0.7 μM PO_4^{3-}) at 20°C into 600-L aerated tanks. During this period, the algae remained healthy.

Description of systems

The experimental system was a completely closed system designed for integrated fish-seaweed culture that consisted of six PVC tanks (Fig. 1). While there was inflow and outflow in the previous aquaculture systems, there was no the input and output of water in this system during the experimental period. One 700-L tank (1.1 m^3) was used for fish culture, one 400-L tank was used for sedimentation, three tanks (200 L each, 0.3 m^2) were used for seaweed cultures, and one 300-L tank was used for pumping (Fig. 1). Coral sands were used as a sandy filter. Seawater was circulated by both gravity and an electric pump from the fish tank into the sedimentation tank and then into the three seaweed tanks, which were arranged in series. The seawater from the seaweed tanks was then returned to the fish tank through the pumping tank (which contained an electric pump). The sedimentation tank was screened for accumulated particles with a sand filter made of coral. The seaweed tanks were screened at each end of outflow to prevent washout of the seaweed. All tanks were shaded with black screen, which reduced irradiance to about 70% of the total sunlight. Irradiance ranged from 0 (at night) to 350 μmol photons m^{-2} s^{-1} (during the day). Water temperature was maintained at 19.5 ± 0.2°C using a cooler. The seawater was recirculated at a water flux of 308.4 ± 5.1 L h^{-1} (3.7 vol d^{-1}). The salinity ranged from 31.6 and 32.0 psu.

To achieve the objectives of this study, the experiments consisted of a fish monoculture system (without

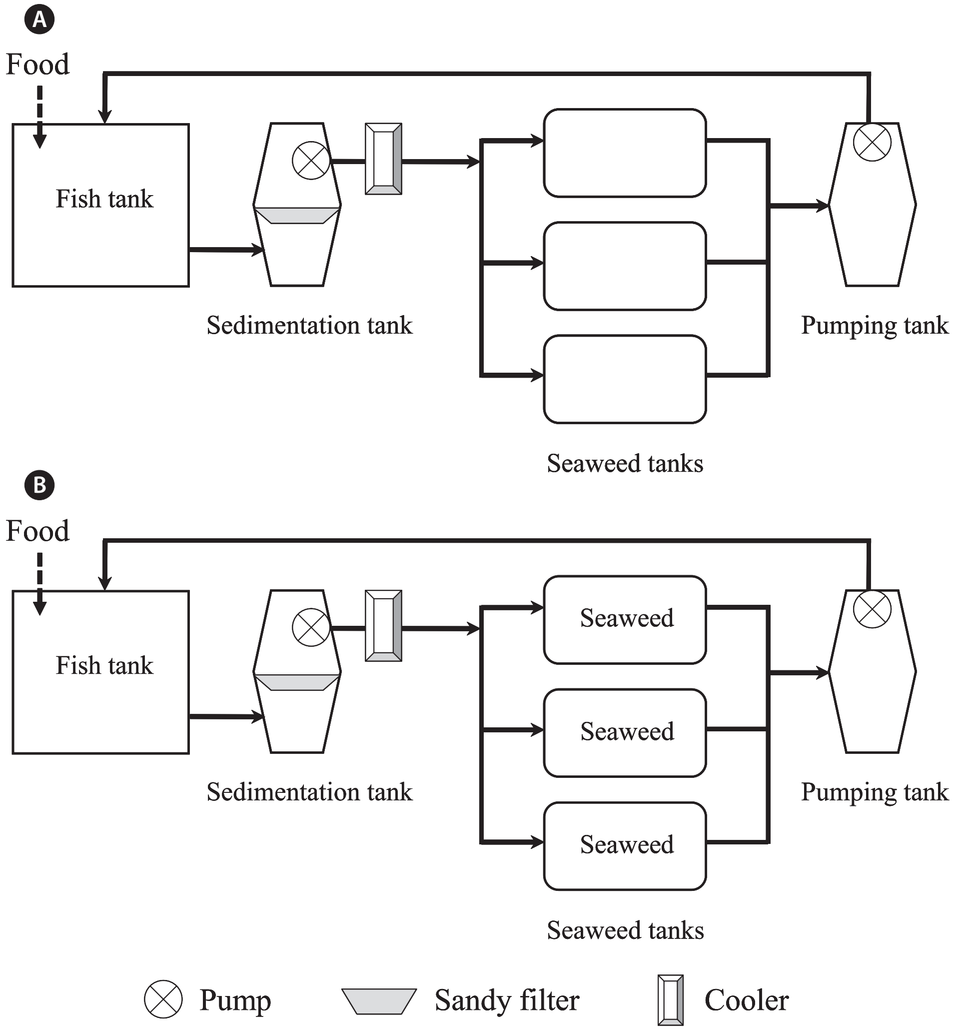

Fig. 1. Schematic diagram of land-based integrated systems (A, fish monoculture system without seaweed; B, fish-seaweed integrated system). Arrows indicate the directions of water flow.

seaweed) (Fig. 1A) and a fish-seaweed integrated system (with seaweed) (Fig. 1B) that were subjected to the same conditions and were conducted simultaneously. The fish monoculture system served as a control system. Black rockfish *Sebastes schlegeli* (approximately 5.0 kg) were stocked in a 700-L aerated tank. In addition, the seaweeds *U. pertusa*, *S. japonica*, and *G. chorda* were stocked at 10 g FW L^{-1} (6.7 kg FW m^{-2}). The fish were fed to satiation daily at 07:00 h. The daily nitrogen and phosphorus inputs in these systems were about 1.26 g and 0.22 g, respectively. The experiments were conducted for two weeks during which time all thanks were drained weekly and were filled with fresh seawater from Gamak Bay. The experiments were conducted in triplicate.

Water sampling and analysis

Water samples were collected from the pumping tank in the fish monoculture and the fish-seaweed integrated systems at 06:00 and 18:00 h every day for seven days to determine the water column inorganic nutrients concentrations (NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-}). The $NO_3^- + NO_2^-$ concentration was determined after running the sample through a column containing copper-coated cadmium, which reduces NO_3^- to NO_2^-. The nutrient concentrations were determined using standard colorimetric techniques according to the methods described by Parsons et al. (1984).

Tissue nutrients content analysis

Tissue samples for carbon (C), nitrogen (N), and phosphorus (P) analysis were collected prior to the experiments and again at the end of each experiment. The samples were then dried at 60°C for 48 h, after which the tissues were ground using a mortar and pestle. Approximately 2-3 mg of ground tissue was placed into a tin to determine the carbon and nitrogen contents using a CHN elemental analyzer (Vario-EL III; Elementar, Hanau, Germany). The tissue P content was analyzed using the molybdovanadophosphate method (Kitson and Mellon 1944) after nitric acid / perchloric acid digestion. The elemental content was calculated on a dry weight basis while elemental ratios were calculated on a mole : mole basis.

Nutrient reduction efficiency calculation

The biofiltering efficiency (%) of seaweed was estimated based on nutrient concentrations in the effluents from the fish monoculture system and the fish-seaweed integrated system using the following equation (Hernández et al. 2005):

$$\text{Biofiltering efficiency (\%)} = (A - B) / A \times 100$$

where A and B are the nutrient concentrations (NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-}) in the effluents of the seaweed tank in the fish monoculture and fish-seaweed integrated systems, respectively. For the calculations, it was assumed that removal by planktonic cells and epiphytes growing on the tank walls were similar in both systems (Hernández et al. 2006, Zhou et al. 2006).

Statistical analysis

The data were tested for normality and homogeneity of variance to determine if they met the assumptions of parametric statistics. However, these assumptions were not satisfied; therefore, nonparametric tests were used. Specifically, the differences in nutrient concentrations in the seaweed tank effluents between the fish monoculture system and fish-seaweed integrated system at the end of the experiment were tested for significance using the Mann-Whitney U-test. Differences in C, N, and P contents and the C : N and N : P ratios in macroalgal tissues between samples taken before and after the experiments were also tested for significance using the Mann-Whitney U-test. Statistical significance was set at the alpha < 0.05 level. All statistical analyses were performed using SPSS ver. 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The nutrient biofiltering efficiency of seaweeds

All nutrient (NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-}) concentrations were significantly (Mann-Whitney U-test, all $p < 0.001$) lower in the fish-seaweed integrated system than in the fish monoculture system. Nutrients concentrations in the fish monoculture and fish-seaweed integrated systems were constant during the first 24-36 h, after which they increased dramatically (Figs 2-4). The NH_4^+ concentrations in the fish monoculture system increased more during the day than during the night, while the NH_4^+ concentrations in the fish-seaweed integrated system usually increased during the night and decreased or increased only slightly during the day (Fig. 2). The NH_4^+ concentrations in the fish monoculture system ranged from 75.6 ± 2.4 (*U. pertusa*) to 92.0 ± 2.5 μM (*S. japonica*) after seven days. The $NO_3^- + NO_2^-$ concentrations in the fish monoculture system increased during the night (Fig. 3). The $NO_3^- + NO_2^-$ concentrations were highest (70.8 ± 0.1 μM) in *U. pertusa* and lowest (55.6 ± 0.3 μM) in *G. chorda*. Additionally, the $NO_3^- + NO_2^-$ concentrations in the fish-seaweed integrated system containing *U. pertusa* and *G. chorda* increased dramatically, whereas those with *S. japonica* showed gradual increases throughout the experimental period. Fig. 4 shows the variation in the PO_4^{3-} concentrations in the fish monoculture and the fish-seaweed integrated systems containing each species during

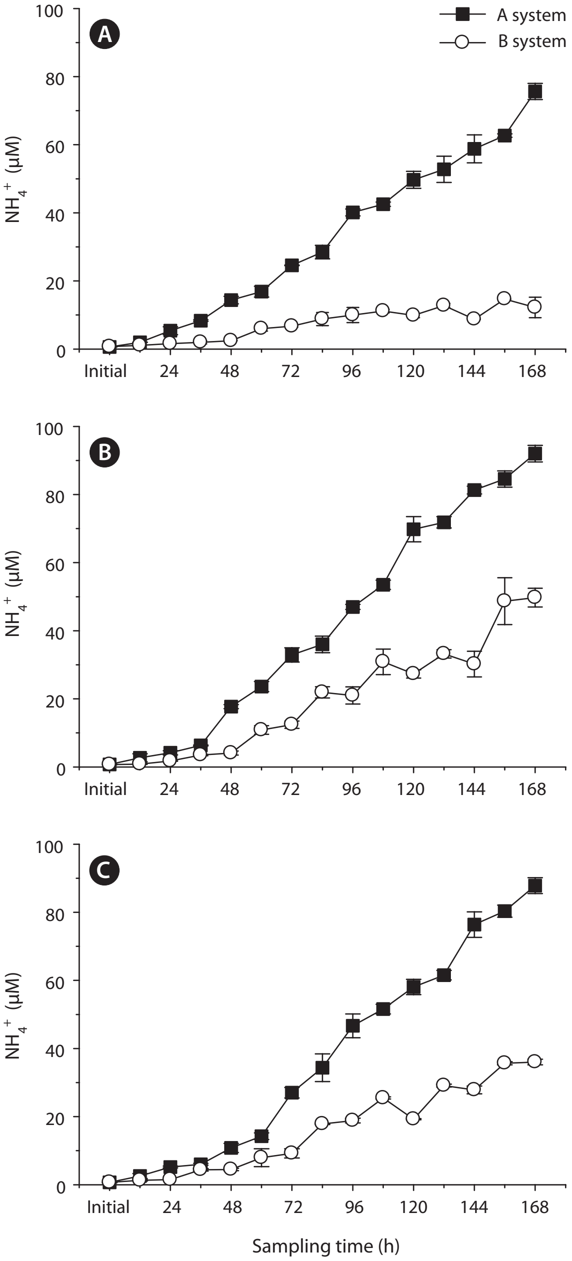

Fig. 2. Temporal changes in NH_4^+ concentration of *Ulva pertusa* (A), *Saccharina japonica* (B) and *Gracilariopsis chorda* (C) in the effluent of seaweed tanks from A system (fish monoculture system) and B system (fish-seaweed integrated system). The data are expressed as the mean ± standard error (n = 3).

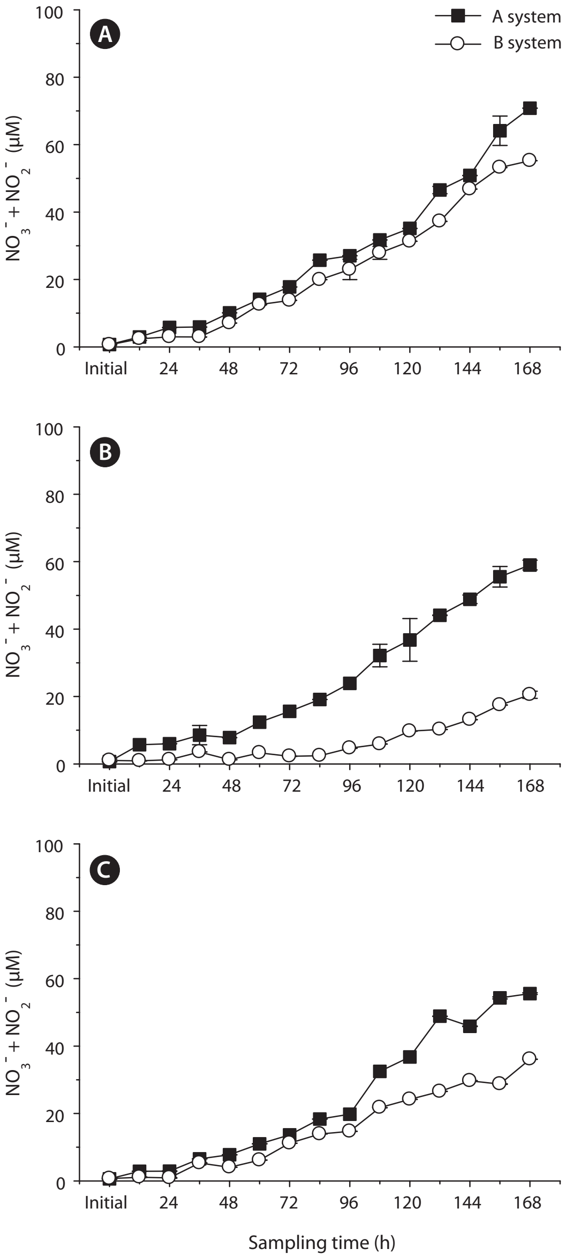

Fig. 3. Temporal changes in the $NO_3^- + NO_2^-$ concentration of *Ulva pertusa* (A), *Saccharina japonica* (B) and *Gracilariopsis chorda* (C) in the effluent of seaweed tanks from A system (fish monoculture system) and B system (fish-seaweed integrated system). The data are expressed as the mean ± standard error (n = 3).

the experimental period. The PO_4^{3-} concentrations in the fish monoculture system were less than 10 μM after seven days. Among the three species, the average PO_4^{3-} concentrations in the fish-seaweed integrated system were approximately 5.0 μM at the end of the experiment.

In this study, the nutrient (NH_4^+, NO_3^- + NO_2^-, and PO_4^{3-}) biofiltering efficiency varied with species, showing high variation during the first 36 h, and then becoming relatively constant with time (Fig. 5). In addition, there were slight differences in total nitrogen biofiltering efficiency among species (Table 1). However, the biofiltering efficiencies of NH_4^+ and NO_3^- + NO_2^- for each species var-

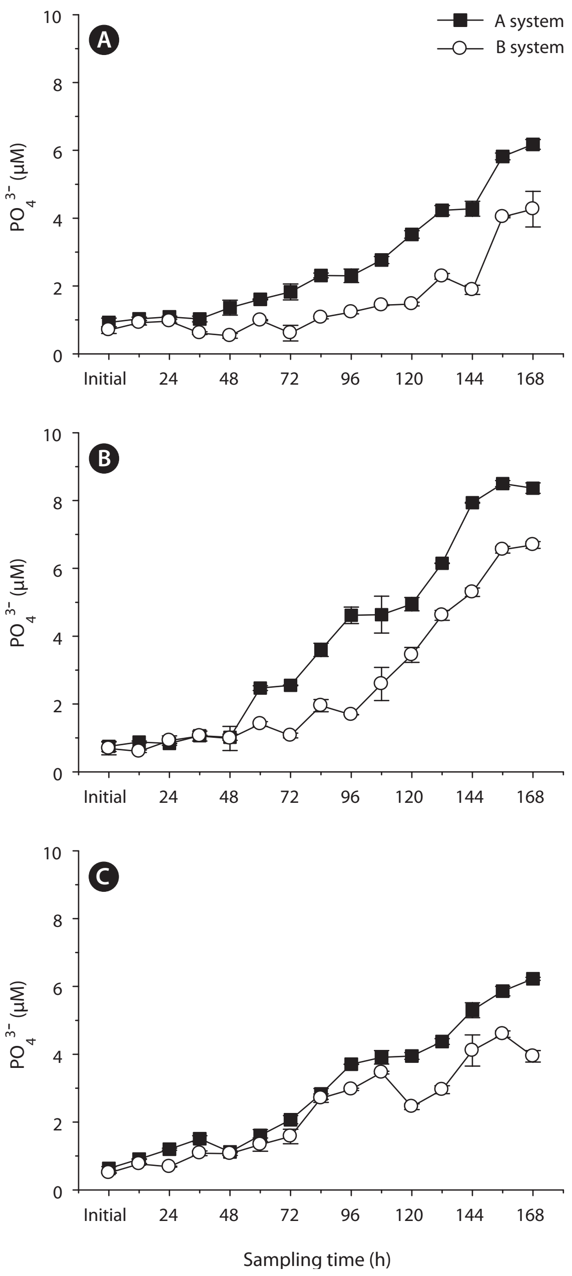

Fig. 4. Temporal changes of PO_4^{3-} concentration of *Ulva pertusa* (A), *Saccharina japonica* (B) and *Gracilariopsis chorda* (C) in the effluent of seaweed tanks from A system (fish monoculture system) and B system (fish-seaweed integrated system). The data are expressed as the mean ± standard error (n = 3).

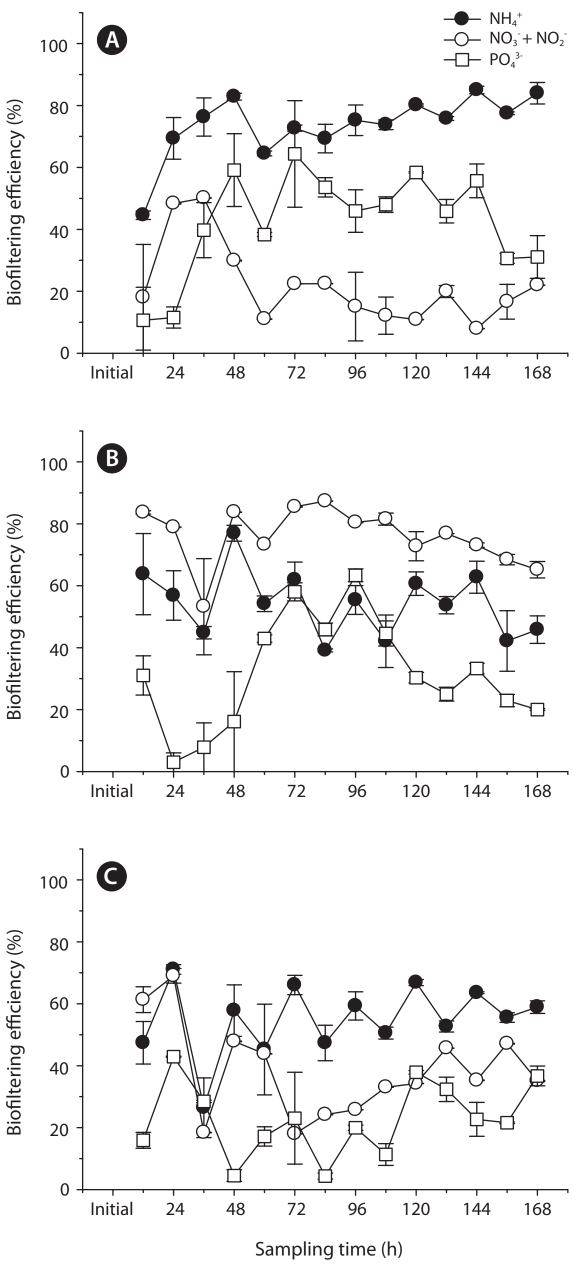

Fig. 5. Nutrient (NH_4^+, NO_3^-+NO_2^-, and PO_4^{3-}) biofiltering efficiency of *Ulva pertusa* (A), *Saccharina japonica* (B) and *Gracilariopsis chorda* (C). The data are expressed as the mean ± standard error (n = 3).

ied greatly. Of the three species investigated, *U. pertusa* showed the highest NH_4^+ biofiltering efficiency during the experimental period whereas its $NO_3^- + NO_2^-$ biofiltering efficiency was very low (Table 1). For *G. chorda*, the NH_4^+ biofiltering efficiency was higher than the $NO_3^- + NO_2^-$ biofiltering efficiency (Table 1). In contrast, *S. japonica* showed the highest $NO_3^- + NO_2^-$ biofiltering efficiency (65.2%) at the end of the experiment. The PO_4^{3-} biofiltering efficiencies of the three species were lower than the total nitrogen efficiency. After seven days, the PO_4^{3-} biofiltering efficiency ranged from 38% for *G. chorda* to 20.2% for *S. japonica*.

Tissue nutrients content

The tissue C content of *S. japonica* decreased significantly by the end of the experiment, while that of *G. chorda* increased significantly (Table 2). However, no differences were observed in the tissue C content of *U. pertusa* between the start and the end of the experiment. The tissue N content of all species increased significantly at the end of the experiment (Table 2). The tissue N content of *U. pertusa* increased by only 3% when compared to the initial value, while that of *G. chorda* increased by 36% higher. Moreover, all species showed significant increases in tissue P content by the end of the experiment (Table 2). Specifically, the tissue P contents of the three species increased by 30 to 55% over their initial values.

The C : N ratios of the three species were significantly lower by the end of the experiment (Table 2). Although *G. chorda* showed a significant increase in tissue C content, a marked decrease in its C : N ratio was observed due to the high increase in tissue N content. In contrast to the consistent C : N ratio decline, changes in N : P ratios were dependent on species (Table 2). The N : P ratio of *S. japonica* at the end of the experiment was lower than at the beginning of the experiment. However, the N : P ratio of *U. pertusa* at the end of the experiment showed increases over their initial values. *G. chorda* showed no difference in the N : P ratio between the start and the end of the experiment.

DISCUSSION

During the last two decades, many studies examined the role of seaweed as a biofilter for removing or reducing mass nutrient sources from seawater effluents in integrated aquaculture systems; however, these studies have usually focused on nitrogen sources (e.g., reviewed by Troell et al. 2003). Indeed, only a few studies have addressed phosphate biofiltration by seaweed, and the data they generated were not sufficient (Troell et al. 1997, Jones et al. 2001). In the present study, we simultaneously measured the nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) and phosphate (PO_4^{3-}) biofiltering efficiencies of seaweed due to the importance of phosphate in recirculating culture systems.

We found that the biofiltration efficiencies of seaweed species showed dial variations, increasing during the

Table 1. Dissolved inorganic nitrogen (DIN; NH_4^+ and $NO_3^- + NO_2^-$) and PO_4^{3-} concentrations of the effluents in seaweed tanks in the fish monoculture system (A) and the fish-seaweed integrated system (B) and the nutrients reduction efficiency by seaweed at the end of the experiments

Species	Nutrient (µM)	A system	B system	Reduction efficiency (%)
Ulva pertusa	DIN	146.4	67.4	54.0
	NH_4^+	75.6	12.2	83.9
	$NO_3^- + NO_2^-$	70.8	55.2	22.0
	PO_4^{3-}	6.2	4.3	30.6
Saccharina japonica	DIN	151.0	70.2	53.5
	NH_4^+	92.0	49.7	46.0
	$NO_3^- + NO_2^-$	59.0	20.5	65.2
	PO_4^{3-}	8.4	6.7	20.2
Gracilariopsis chorda	DIN	143.5	72.1	49.8
	NH_4^+	87.9	36.0	59.0
	$NO_3^- + NO_2^-$	55.6	36.1	35.1
	PO_4^{3-}	6.3	3.9	38.1

Data are the means (n = 3).

Table 2. Macroalgal tissue C and N contents, and C : N and N : P ratios at the beginning and end of the experiments

Species	Tissue C content (%)		p-value	Tissue N content (%)		p-value	Tissue P content (%)		p-value	C : N ratio		p-value	N : P ratio		p-value
	Initial	Final		Initial	Final		Initial	Final		Initial	Final		Initial	Final	
Ulva pertusa	34.2 ± 0.2	34.0 ± 0.1	0.241	2.32 ± 0.03	2.39 ± 0.01	0.046	0.10 ± 0.01	0.13 ± 0.01	0.001	17.2 ± 0.1	16.6 ± 0.1	0.009	52.7 ± 0.2	43.4 ± 0.6	0.001
Saccharina japonica	34.5 ± 0.2	32.9 ± 0.2	0.001	1.29 ± 0.01	1.56 ± 0.07	0.020	0.20 ± 0.01	0.31 ± 0.02	0.003	31.3 ± 0.1	24.6 ± 0.8	0.007	14.8 ± 0.3	11.8 ± 1.1	0.025
Gracilariopsis chorda	36.5 ± 0.3	41.1 ± 0.3	0.001	2.15 ± 0.08	2.93 ± 0.13	0.017	0.17 ± 0.01	0.23 ± 0.01	0.004	19.8 ± 0.6	16.5 ± 0.6	0.026	28.9 ± 2.1	30.0 ± 2.3	0.386

Data are the mean ± standard error (n = 6). C and N contents and C : N and N : P ratio in macroalgal tissues between at the initial and at the end of the experiments were tested for significance using the Mann-Whitney U test.

daytime and decreasing during the night. This may be explained by the feeding pattern of fish and the effects of irradiance on the response of seaweed growth. Hernández et al. (2006) found that inflow NH_4^+ concentrations, which were lower in the morning and higher in the afternoon, reflected the feeding period. In addition, the nutrient uptake rates of seaweed are positively affected by irradiance before they are saturated with irradiance (Nanba et al. 2005, Pereira et al. 2006). Therefore, seaweed is capable of taking up more nutrients from water for growth (Kang et al. 2008). Consequently, the daily patterns of nutrient biofiltering efficiency were controlled by two factors, feeding period and the effects of irradiance on seaweed growth.

In the present study, the total inorganic nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) biofiltering efficiencies of the three species evaluated were approximately 50% at the end of the experiments. *U. pertusa* showed the highest NH_4^+ biofiltering efficiency, which was similar to findings of a study conducted by Hernández et al. (2005) who reported that the nitrogen efficiency of *U. rotundata* reached to 85% in a 600-L tank. However, *G. chorda* exhibited relatively low NH_4^+ biofiltering efficiency when compared to the results (approximately 75%) of a study that employed a 5 kg m^{-2} stocking density and 1,400 L tank (Matos et al. 2006). In this study, the kelp species, *S. japonica*, showed NH_4^+ biofiltering efficiencies greater than 40%, suggesting that they could be used as a biofilter in a fish-seaweed integrated system.

Nitrogen originating from fish excretions is usually available in the form of NH_4^+ in aquaculture systems (Matos et al. 2006). However, nitrification (oxidizing NH_4^+ to form $NO_3^- + NO_2^-$) by biological filters allows various forms of nitrogen to be present in oxygen-rich waters or in recirculating culture systems (Troell et al. 2003). Therefore, a large amount of NO_3^- and NO_2^-, as well as NH_4^+, may have been present in this system. In the present study, $NO_3^- + NO_2^-$ concentrations increased throughout the experimental period. This was likely due to the structure of the coral sand used as a filter. Coral sands with high permeability may permit water flow through pore spaces in the upper sediment layers (Wild et al. 2009), which would allow a high rate of nitrification.

According to previous literature data, most seaweed species showed that uptake rates of NH_4^+ exceeded those of NO_3^- under natural environmental conditions (Neori et al. 1996, Ahn et al. 1998). These results were similar to those previously reported for *Ulva* and *Gracilaria* genus (Krom et al. 1995, Naldi and Wheeler 2002). *U. lactuca* strongly preferred NH_4^+ over $NO_3^- + NO_2^-$. Additionally, *Gracilaria* species exhibited higher NH_4^+ uptake than $NO_3^- + NO_2^-$ uptake. Specifically, *U. pertusa* showed the highest uptake rate of the three seaweed species and made intense use of NH_4^+. In contrast, *S. japonica* showed high $NO_3^- + NO_2^-$ removal efficiencies when compared to NH_4^+ removal efficiencies (Table 1). Specifically, this species took up $NO_3^- + NO_2^-$ preferentially over NH_4^+. This was an interesting result for the metabolic energy efficiency of seaweed species because NH_4^+ can be directly incorporated into amino acids (Ahn et al. 1998). Harrison et al. (1986) reported that *S. groenlandica* utilized NH_4^+ and $NO_3^- + NO_2^-$ simultaneously and that the uptake rates were identical. The use of both NH_4^+ and $NO_3^- + NO_2^-$ at one time may be profitable to kelp that require large amounts of nitrogen per unit time (Thomas and Harrison 1987). Moreover, kelp have a larger storage capacity than other seaweeds and therefore probably receive a greater advantage than other seaweed species when making use of NO_3^- in the water column (Touchette and Burkholder 2000). Therefore, we recommend that effluent standards from fish farms should establish total nitrogen concentrations that include NO_3^- and NH_4^+, and that the selection of seaweed species for use as a biofilter should involve consideration of the nitrogen forms found in the effluents.

Phosphorus is not harmful to fish but is a major nutrient involved in controlling seaweed growth and contributes to downstream eutrophication (Neori et al. 1996). Jones et al. (2001) reported that *Gracilaria edulis* cultivated at a stocking density of 20 g FW L^{-1} into 5 L tanks showed a 47% PO_4^{3-} biofiltering efficiency. *Gracilaria* cultivated at 1.5 kg m^{-2} in a 500 L tank exhibited a 32% of PO_4^{3-} biofiltering efficiency during the study period (Buschmann et al. 1996). For all species, the biofiltering efficiency for PO_4^{3-} was lower than that of the total nitrogen efficiency, and ranged from 20.2 to 38.1%. These results were similar to those reported in the previous data described above (Buschmann et al. 1996, Jones et al. 2001). Therefore, these suggest that the species investigated in the present study are suitable candidates for use as biofilters in integrated aquaculture systems.

Tissue N and P contents in three species increased quickly and continued to increase throughout the study period in response to the high nitrogen concentrations in the fish tank effluents. These results indicate that seaweed growth is not limited by inorganic nutrients. Despite the increase of tissue N contents in all species, the tissue N contents were very low (1.56-2.93%). This can be explained by the period for which the seaweed was exposed to high nutrients. In this study, the tissue N contents were analyzed after seven days, suggesting that the experimental period was not sufficient to achieve high tissue N contents. Assuming that the tissue N contents of *Gracilaria* and *Ulva* cultured in fish effluents increased to 5-7% of their total biomass (He et al. 2008, Kang et al. 2009), these species are still able to store large amounts of nitrogen gleaned from the effluent and serve as biofilters.

Hernández et al. (2002) reported that the C : N ratio of macroalgal tissues might control differences in the nutrient removal efficiency. Therefore, the C : N ratios are a powerful index of the physiological status of seaweed (Vergara et al. 1993) and could be used as an indication of seaweed healthy status. Generally, C : N ratios show low values when nitrogen is abundant and increase when nitrogen supply is limited (Lahaye et al. 1995, Gómez Pinchetti et al. 1998). In the present study, the C : N ratios of all species decreased significantly by the end of the experiment due to the increase in tissue N contents. Moreover, the change in the N : P ratio varied with seaweed species. Tissue N contents increased faster than tissue P contents in *U. pertusa*, and *S. japonica*, leading to declines in the N : P ratios. However, the N : P ratio of *G. chorda* did not change at the end of the experiments due to relative increase in tissue P content. These findings suggest that variations in the N : P ratio may reflect inherent species-specific differences in nitrogen and phosphate uptake rates.

In conclusion, three species (*U. pertusa*, *S. japonica* and *G. chorda*) showed high nitrogen (NH_4^+ and $NO_3^- + NO_2^-$) and PO_4^{3-} biofiltering efficiency (approximately 50%), indicating that they could be used as biofilters in the integrated aquaculture system. In particular, *S. japonica* exhibited a higher preference for $NO_3^- + NO_2^-$ than for NH_4^+. These findings suggest that the choice of a biofilter seaweed species should be made with consideration of the N forms expelled in the effluents. Additionally, the co-culture of two seaweed species that showed nitrogen source-specific biofiltration as a biofilter is a reasonable solution for reducing nutrient from the effluents.

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