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Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 2. Ammonium

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Abstract

Three estuarine macroalgae (*Ulva rotundata*, *Enteromorpha intestinalis*, *Gracilaria gracilis*) of economic potential were cultivated in the laboratory to assess their biofiltering capacities for ammonium in waste effluents from a sea bass (*Dicentrarchus labrax*) cultivation tank. The study was developed to investigate the functioning of N nutrition of the three species. At low water flow (< 2 volumes d^{-1}) the three species stripped efficiently the ammonium dissolved in the waste water from the fish tank, with a minimum biofiltering efficiency estimate of 61% in unstarved cultures of *G. gracilis* at a water flow of 2 volumes d^{-1}. Maximum velocity for ammonium uptake (89.0 μmol NH_4^+ g^{-1} dry wt h^{-1}) was found in *U. rotundata*, whereas *G. gracilis* showed the highest affinity for this nutrient. The net ammonium uptake rate was significantly affected by the water flow, being greatest at the highest flow assayed (2 volumes d^{-1}). Variations of tissue N and C:N ratios during a flow-through experiment suggested that N was not limiting macroalgal growth. However, when ammonium was supplied at a flow rate of 0.5 volumes d^{-1}, specially in a three-stage design, the marked reduction in tissue N and the biomass C:N:P ratios suggested a more general nutrient deficiency. A significant correlation was found between growth rates and the N biomass gained in the cultures. The three-stage design under low water flow (0.5 volumes d^{-1}) showed that the highest ammonium uptake rates (up to 80.9 μmol NH_4^+ g^{-1} dry wt d^{-1} in *U. rotundata*) were found in the first stage, with decreasing rates in the following ones. As a result, low increments or even losses of total N biomass in these stages were found, suggesting that ammonium was excreted from the algae. We conclude that these species present a potential ability to biofilter the ammonium dissolved in waste water from a *D. labrax* cultivation tank, suggesting that scaling up the biofiltration designs, future practises using these macroalgae may be implemented in the local fish farms, resulting in both environmental and economical advantages.

Introduction

There have been several studies reporting the benefits of integrating the production of macroalgae with the production of fish or invertebrates to remove nutrients from waste water effluents (e.g. Jiménez del Río et al. (1996) and Troell et al. (1999)). Dissolved ammonium can be biofiltered to a high extent in integrated aquaculture practises. For instance, the rhodophyta *Gracilaria chilensis* was able to remove 50% of ammonium in winter, increasing to 90–95% in spring in a farming of salmon and *Gracilaria* (Buschmann et al. 1996). The treatment of shrimp effluents by oyster filtration and *Gracilaria edulis*, resulted in a substantial reduction of ammonium discharge levels (Jones et al. 2001). In addition, other macroalgal species have been proposed to develop integrated systems for ammonium stripping in effluents from intensive fish farms (e.g. Neori et al. (1998) and Chopin et al. (2000, 2001)).

In the previous laboratory study, Martínez-Aragón et al. (2002) showed that *Ulva rotundata*, *Enteromor-*

pha intestinalis and *Gracilaria gracilis*, three estuarine macroalgae, biofiltered efficiently the dissolved phosphate in effluents from a sea bass cultivation tank, when water was circulated at low flow rates. An important issue for optimal scale-up of these laboratory systems to the field is to have a detailed understanding of the physiology of the selected species, which can be complex and necessitate a compromise between apparently conflicting aims (e.g. biomass production *versus* bioremediation efficiency: Chopin et al. (2001)). The aim of the present study was to assess the capability of the same species to biofilter ammonium, the main dissolved nitrogenous compound excreted by the sea bass, as this species does not excrete significant amounts of nitrite or nitrate (Dosdat et al. 1996; Lemarié et al. 1998).

Materials and methods

Samples of *U. rotundata*, *E. intestinalis* and *G. gracilis* were collected and precultured as previously (Martínez-Aragón et al. 2002).

Experimental set-up

Ammonium uptake experiments were carried out as in the previous study (Martínez-Aragón et al. 2002). The excretory products from the fish and the uneaten feed increased the mean ammonium concentration in the waste water by about 15% (34 to 39 μM), although concentrations up to 62 μM were measured. In contrast, nitrate concentration always remained below analytic detection limits. Uptake rates were expressed as μmol NH_4^+ g dry wt^{-1} h^{-1}. Kinetic uptake parameters were calculated from the Michaelis-Menten equation.

The biofiltering capacity of the species was tested as in a previous study (Martínez-Aragón et al. 2002). A flow-through design was conducted under three different flow rates (0.5, 1, and 2; volumes d^{-1}). This experiment was run with and without a previous step of algal maintenance under nutrient deprivation (6 days in oligotrophic seawater collected offshore). The biofiltering capacity of the species was also tested in a three-stage design.

After each experiment, the algae were dried at 60 °C for 3 days. The dried tissue was ground and stored in vials for C and N analysis. The net rate of ammonium uptake for a given time was expressed as μmol NH_4^+ g^{-1} dry wt d^{-1}. The integrated rates of nutrient uptake were computed as the sum of the uptake rates on each time interval divided by the time of the experiment to allow comparison among the experiments.

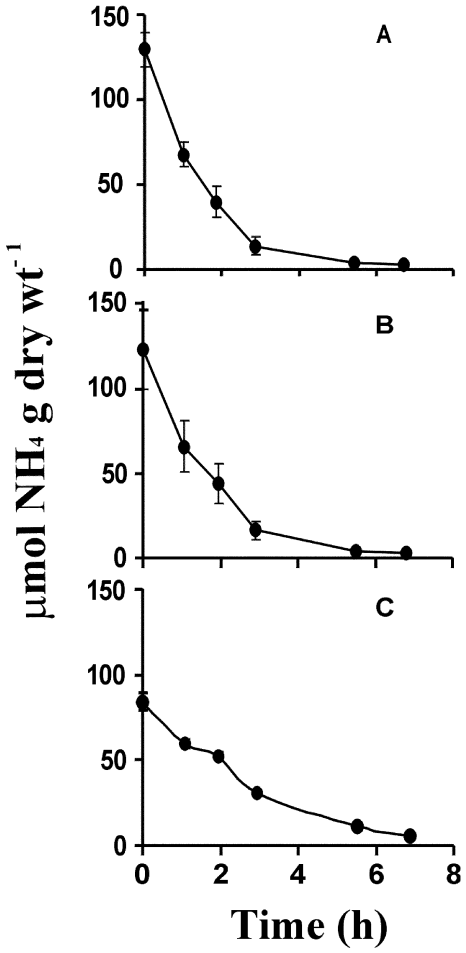

Figure 1. Kinetics of ammonium disappearance from the medium. A) *Ulva rotundata*; B) *Enteromorpha intestinalis*; C) *Gracilaria gracilis*. Data are means ± SD (n = 3).

Table 1. Ammonium uptake kinetic parameters of the three macroalgal species based on the Michaelis-Menten model for uptake kinetics. V_{max} (maximum uptake rate, μmol g^{-1} dry wt h^{-1}), K_s (half-saturation value, μM) and V_{max}/K_s ratio, an estimator of the uptake affinity. Data are means ± SD (n = 3).

Species	V_{max}	K_s	V_{max}/K_s
Ulva rotundata	89.0 ± 25.2	20.1 ± 4.60	4.43
Enteromorpha intestinalis	79.5 ± 9.41	21.3 ± 0.29	3.73
Gracilaria gracilis	21.3 ± 1.98	11.3 ± 1.50	1.88

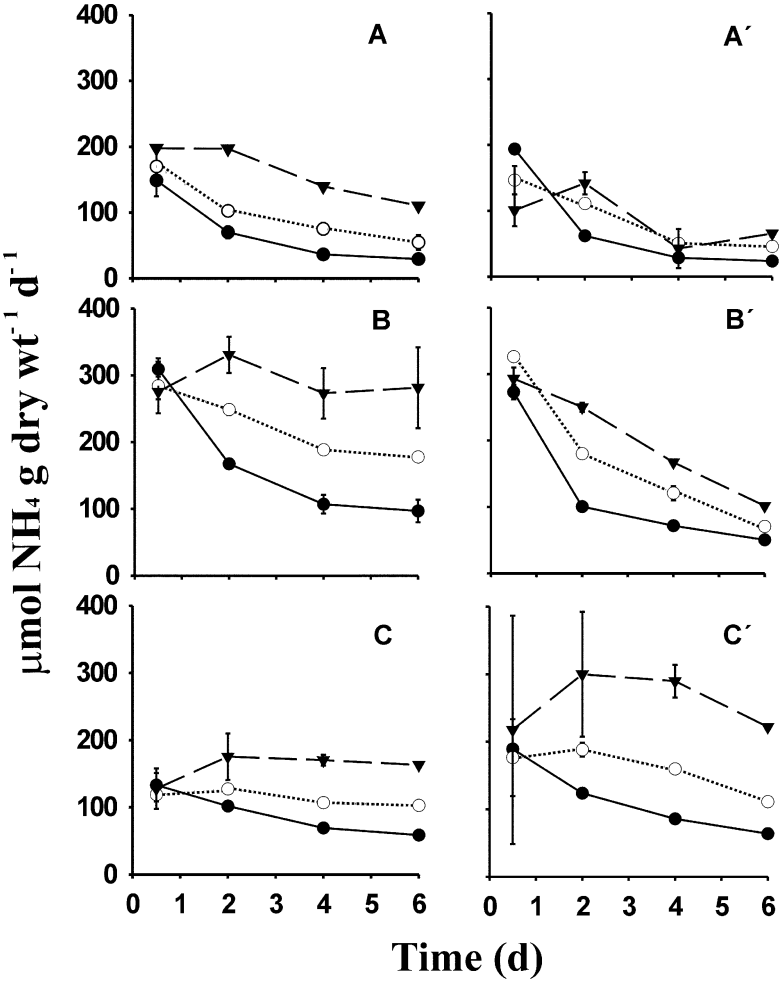

Figure 2. Net dissolved ammonium uptake rates versus time at water flow of 0.5 (●), 1 (^), and 2 (▼) volumes d^{-1} without a previous step of algae maintenance under nutrient deprivation (normal caps) or with such step of 6 days in oligotrophic sea water (prime caps). A-A′) *Ulva rotundata*; B-B′) *Enteromorpha intestinalis*; C-C′) *Gracilaria gracilis*. Data are means ± SD (n = 2 – 3).

Chemical analysis

Ammonium and nitrate were analysed according to Grasshoff et al. (1983) through a flow injection analysis (Tovar et al. 2000). Water samples were previously filtered through Whatman GF-F filters. Tissue C and N was determined using a Perkin-Elmer 240 CNH elemental analyser.

Statistics

The overall effects of the previous step of macroalgal maintenance under nutrient deprivation and flow rates on integrated rates of ammonium uptake were analysed by a two-factor ANOVA (model I). Multiple post hoc comparisons among means were tested by the Tukey test (Zar 1984). Correlation between macroalgal growth rates and the increase of N biomass during biofiltration cultures was analysed by the Pearson correlation coefficient. In all cases, the null hypothesis was rejected at the 5% significance level.

Results

U. rotundata, *E. intestinalis* and *G. gracilis* removed efficiently the ammonium dissolved in waste waters from the fish tank (Figure 1). After 7 h, most of the ammonium was taken up by the algae, as nutrient concentration was constant in the control cultures (data not shown). *U. rotundata* and *E. intestinalis* showed the highest ammonium disappearance

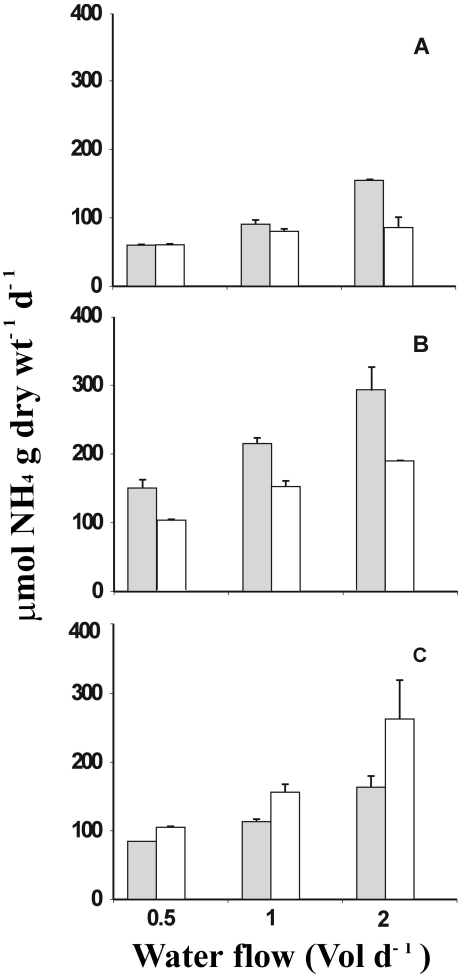

Figure 3. Integrated dissolved ammonium uptake rates at different flow rates. Full bars represent rates measured in unstarved cultures. Empty bars represent rates under a previous step of 6 days in oligotrophic sea water. A) *Ulva rotundata*; B) *Enteromorpha intestinalis*; C) *Gracilaria gracilis*. Data are means ± SD (n = 2, 3).

(97.7%), whereas *G. gracilis* removed 93.2% of the nutrient during the incubation (Figure 1C).

The minimum uptake rate of ammonium occurred in *G. gracilis* (Table 1). In *E. intestinalis*, and especially in *U. rotundata*, V_{max} took place at ammonium concentrations much higher than those measured in the waste water used in the subsequent experiments (data not shown). The half saturation constant for uptake (K_s) was also lowest in *G. gracilis* (Table 1). However, this species also showed the lowest efficiency of ammonium uptake, defined by the ratio V_{max}/K_s, whereas the highest efficiency was estimated for *U. rotundata*.

The net ammonium uptake rates in the flow-through design decreased during the experimental period and were usually affected by seawater flow, being greater under 2 volumes d^{-1}, with the lower ones under 0.5 volumes d^{-1} (Figure 2). The lowest uptake rates were estimated in *U. rotundata*, especially when the algae were previously incubated under nutrient deprivation. The integrated ammonium uptake rates increased significantly with the water flow (Figure 3), as shown by the factorial ANOVA (Table 2) and the post hoc means comparison (data not shown). The effect of previous maintenance with or without nutrient deprivation on the net ammonium uptake was variable (Figure 3, Table 2): no effect (*U. rotundata*), significantly enhanced uptake rates under ammonium deprivation (*G. gracilis*) or the opposite effect (*E. intestinalis*).

Table 3 shows the ammonium uptake efficiency of the species when water from the fish tank was circulated at 0.5, 1 and 2 volumes d^{-1} flow rates. Ammonium was biofiltered at a high percentage in all cultures, with outflow concentrations decreasing at least 60% at the end of the experiments. Overall, the biofiltration efficiency decreased with the water flow and was usually greater when the algae were previously maintained under nutrient deprivation. These algae also grew at a lower rate (Martínez-Aragón et al. 2002). The higher filtration efficiency was found in *E. intestinalis*, especially at a flow of 0.5 volumes d^{-1}, where the nutrient was virtually extinguished in the water outflowing at the end of the experiment.

Changes in tissue N concentration are shown in Table 4. The N content at the onset of the flow-through experiment diminished when the algae were preincubated under nutrient deprivation. After the experiment, unstarved cultures of *U. rotundata* and *E. intestinalis* evidenced a moderate decrease in tissue N, despite the high production of macroalgal tissue. In contrast, *G. gracilis* increased its tissue N concentration under all flow rates. The results were different when the algae were previously cultured under nutrient deprivation. After the experiments, lower tissue N contents were measured in *U. rotundata* and *G. gracilis*, but a pronounced increase in this element up to 29% was measured in *E. intestinalis*. In all biofiltration designs, the net N biomass increased in the cultures due to the increment of algal biomass (Table 4). The N biomass produced was similar to the total ammonium input to the system (data not shown). Moreover, when data from the three species were pooled, there was a significant correlation between growth

Table 2. Summary of the analysis of variance showing the effect of the cultivation with or without a previous maintenance under nutrient deprivation and flow rates on the integrated rates of ammonium uptake of the three macroalgal species. DF: Degrees of freedom; MS: Mean square.

	Factors	DF	MS	F-value	p
Ulva rotundata	Preincubation	1	515	0.181	0.680
	Water flow	2	17.7	6.20	0.018
	Interaction	2	980	0.343	0.718
Enteromorpha intestinalis	Preincubation	1	24.2	38.8	< 0.01
	Water flow	2	9.52	15.3	< 0.01
	Interaction	2	2.07	3.32	0.079
Gracilaria gracilis	Preincubation	1	4.10	26.1	< 0.01
	Water flow	2	8.89	56.6	< 0.01
	Interaction	2	193	1.23	0.330

Table 3. Mean NH_4 concentration at the inflow and outflow of the cultures under three different flow rates and percentage of biofiltration at the end of the flow-through experiment (day 7). Experimental designs were run with (starved cultures) and without (unstarved cultures) a previous step of algae maintenance under nutrient deprivation. Data are mean of three replicates ± SD.

Species	Water flow (volumes d^{-1})	Unstarved cultures NH_4^+ (μM)		Biofiltration efficiency (%)	Starved cultures NH_4^+ (μM)		Biofiltration efficiency (%)
		Inflow	Outflow		Inflow	Outflow	
Ulva rotundata	0.5	32.2 ± 6.42	3.94 ± 0.83	87.8	24.8 ± 8.52	2.91 ± 0.69	88.2
	1	32.2 ± 6.42	5.68 ± 0.77	82.3	24.8 ± 8.52	3.03 ± 0.20	87.8
	2	32.2 ± 6.42	5.34 ± 0.49	83.4	24.8 ± 8.52	4.92 ± 1.54	80.1
Enteromorpha intestinalis	0.5	43.0 ± 5.98	2.63 ± 0.78	93.9	22.2 ± 5.87	0.3 ± 0.10	99.9
	1	43.0 ± 5.98	3.82 ± 1.26	91.1	22.2 ± 5.87	1.43 ± 0.56	93.6
	2	43.0 ± 5.98	8.13 ± 1.19	81.1	22.2 ± 5.87	1.01 ± 0.49	95.5
Gracilaria gracilis	0.5	35.8 ± 4.99	4.82 ± 2.88	86.5	33.0 ± 6.12	3.56 ± 0.87	89.2
	1	35.8 ± 4.99	9.76 ± 3.11	72.7	33.0 ± 6.12	3.19 ± 0.83	90.3
	2	35.8 ± 4.99	13.9 ± 2.03	61.0	33.0 ± 6.12	3.90 ± 0.38	88.2

rates and the net N biomass gained in cultures ($r = 0.75$, $p = 0.0002$; Figure 4). Furthermore, this increase in N biomass was significantly correlated with the tissue P gained (Martínez-Aragón et al. 2002) in the biofiltration experiments ($r = 0.66$, $p = 0.023$).

The fishpond effluent generally increased the biomass C:N and N:P ratio during the experiments at the three water exchange treatments (Table 5). The tissue C:N ratio of the species diminished with the increase in water flow. This was attributed to a greater ammonium supply, which led to a greater N biomass gained in the cultures (Table 4). The initial biomass C:N ratio increased when the algae were maintained previously under nutrient deprivation (Table 5). This generally caused slightly higher C:N ratios at the end of the experiments, as compared with the unstarved cultures. The marked increase of the biomass N:P ratio in all cultures suggested that P, rather than N, limited macroalgal growth.

The integrated rates of ammonium uptake in the three-stage design are shown in Figure 5. The greatest rate of ammonium uptake was always measured in the first stage. This culture reduced markedly the concentration in the outflow water, as the algae were previously maintained under nutrient deprivation. As a result, much lower or even negligible integrated rates of ammonium uptake were measured in the following stages.

Table 6 shows the ammonium uptake efficiency of the macroalgae in the three-stage design. Ammonium was biofiltered at high efficiencies in the cultures, especially in the initial stage, where the highest growth rates were measured (Martínez-Aragón et al. 2002). The three species showed similar biofiltration capac-

Table 4. Tissue N concentrations at the initial and at the end of the experiments, % variation in tissue N and total N biomass gained during the flow-through biofiltration experiments performed under three different flow rates. Experimental designs were run with (starved cultures) and without (unstarved cultures) a previous step of algae maintenance under nutrient deprivation. Tissue N data were obtained from a pooled sample of three thalli.

Species	Water flow (volumes d^{-1})	Unstarved cultures Tissue N (% dry wt)		% variation per dry wt	mg N gained in culture	Starved cultures Tissue N (% dry wt)		% variation per dry wt	mg N gained in culture
		Initial	Final			Initial	Final		
Ulva rotundata	0.5	3.06	1.35	−55.9	6.64	2.86	1.57	−45.4	2.92
	1	3.06	2.07	−32.4	17.6	2.86	2.51	−12.2	9.82
	2	3.06	2.32	−24.2	19.0	2.86	2.62	−8.36	12.6
Enteromorpha intestinalis	0.5	3.35	2.75	−17.9	5.25	2.30	2.77	20.3	5.36
	1	3.35	2.62	−21.9	4.0	2.30	2.66	15.5	5.94
	2	3.35	3.03	−9.72	11.0	2.30	2.99	29.7	15.5
Gracilaria gracilis	0.5	3.68	3.90	5.94	10.4	3.43	2.45	−28.5	0.569
	1	3.68	4.01	8.90	9.40	3.43	2.75	−19.6	3.93
	2	3.68	3.78	2.52	8.95	3.43	2.86	−16.6	3.87

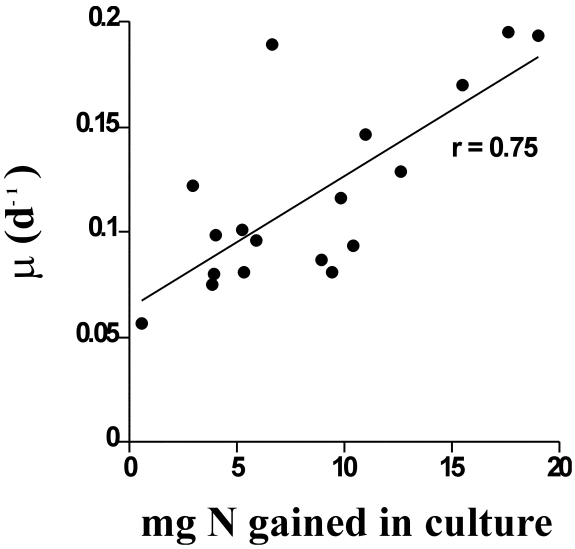

Figure 4. Correlation between growth rate and net N biomass gained in cultures during the flow-through biofiltration experiments (r = 0.75, p = 0.0002).

ities and ammonium was further biofiltered in the following stages. In *G. gracilis*, ammonium concentration increased as water left the second stage and circulated through the last one (as shown by the increase in ammonium concentration; Table 6), suggesting that some ammonium was excreted from the algae.

Changes in tissue N concentration are shown in Table 7. Unlike the previous biofiltration design, the three species showed a marked decrease in tissue N after the three-stage experiment, with percentages of tissue N loss up to 51% in *G. gracilis*. The low water flow (0.5 volumes d^{-1}) and the previous nutrient deprivation of the algae led to similar increments in the net N biomass in the first stage. However, the high ammonium uptake measured in this culture led to lower concentrations in the inflow of the following stages, which subsequently caused lower increase (or even losses) of N biomass. In fact, thalli in some of the following stages at the end of the experiment began to deteriorate. The decrease in the ammonium supply as water circulated through the stages caused an enhancement in the biomass C:N ratios (Table 8). This enhancement was alleviated if N was lost from the tissues during the experiment, as noticed in the last stage of *E. intestinalis* and *G. gracilis* cultures (Tables 7 and 8). Again, the increase in the biomass N:P ratios suggested that P, rather than N, limited growth. However, the tissue N:P ratio at the end of the experiment increased as water circulated through

Table 5. Tissue C:N and N:P ratios at the initial and end of the flow-through biofiltration experiments performed under three different flow rates. Experimental designs were run with (starved cultures) and without (unstarved cultures) a previous step of algal maintenance under nutrient deprivation. C and N data were obtained from a pooled sample of three thalli. Tissue P data from Martínez-Aragón et al. (2002).

Species	Water flow (volumes d^{-1})	Unstarved cultures				Starved cultures			
		C:N ratio		N:P ratio		C:N ratio		N:P ratio	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
Ulva rotundata	0.5	8.41	17.3	40.2	62.3	9.57	16.4	56.5	50.7
	1	8.41	11.8	40.2	57.4	9.57	10.6	56.5	70.3
	2	8.41	9.98	40.2	59.1	9.57	10.0	56.5	55.0
Enteromorpha intestinalis	0.5	8.08	10.3	49.4	50.8	10.2	10.6	39.3	51.6
	1	8.08	10.5	49.4	71.5	10.2	10.9	39.3	64.7
	2	8.08	9.52	49.4	62.7	10.2	9.78	39.3	59.3
Gracilaria gracilis	0.5	8.32	8.30	27.8	42.5	9.56	13.0	28.6	32.5
	1	8.32	7.81	27.8	37.8	9.56	10.7	28.6	32.0
	2	8.32	8.23	27.8	49.7	9.56	8.85	28.6	32.7

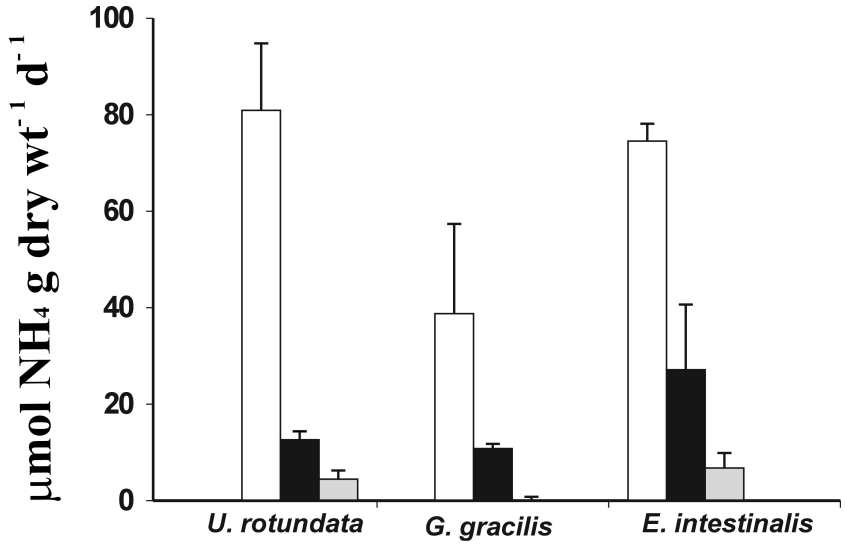

Figure 5. Integrated ammonium uptake rates of the species in the three-stage biofiltration design. White bars: first stage. Black bars: second stage. Grey bars: third stage. Data are means ± SD (n = 2, 3).

Table 6. Mean NH_4 concentration at the inflow and outflow of the three-stage biofiltration design at water flow of 0.5 volumes d^{-1} and NH_4^+ biofiltration efficiency of the three species at the end of the experiments. The accumulated NH_4^+ biofiltration estimated at the outflow of each stage is given in brackets. Data are mean of three replicates ± SD.

Species	Biofiltration stage	NH_4 (μM)		NH_4 filtration efficiency (%)
		Inflow	Outflow	
Ulva rotundata	1	50.6 ± 4.52	5.15 ± 3.32	89.8
	2	5.15 ± 3.32	2.86 ± 0.63	44.5 (94.3)
	3	2.86 ± 0.63	1.74 ± 0.22	39.2 (96.5)
Enteromorpha intestinalis	1	31.3 ± 4.83	1.62 ± 0.53	94.8
	2	1.62 ± 0.53	1.11 ± 0.17	31.3 (96.4)
	3	1.11 ± 0.17	1.03 ± 0.092	6.91 (96.7)
Gracilaria gracilis	1	35.9 ± 7.52	3.24 ± 0.78	91.0
	2	3.24 ± 0.78	3.0 ± 0.66	7.21 (91.6)
	3	3.0 ± 0.66	3.89 ± 1.10	–29.5* (89.2)

*Mean outflow ammonium concentration was higher than inflow concentration

Table 7. Tissue N contents at the initial and at the end of the experiments, percentage loss in tissue N content and total N biomass gained during the three-stage biofiltration design. Tissue N data from a pooled sample of three separate algae.

Species	Biofiltration stage	Tissue N (% dry wt)		% loss per dry wt	mg N gained in culture
		Initial	Final		
Ulva rotundata	1	2.91	1.87	35.7	3.01
	2	1.07	0.81	25.2	0.614
	3	1.27	0.74	41.6	–2.04
Enteromorpha intestinalis	1	3.23	2.10	35.0	6.20
	2	2.86	1.62	43.2	–0.813
	3	2.64	2.0	46.9	0.386
Gracilaria gracilis	1	3.43	2.09	39.0	2.67
	2	2.50	1.21	51.5	–2.32
	3	3.41	1.92	43.8	–2.93

Table 8. Biomass C:N and N:P ratios at the initial and at the end of the three-stage biofiltration design. C and N data were obtained from a pooled sample of three thalli. Tissue P data from Martínez-Aragón et al. (2002).

Species	Biofiltration stage	C:N ratio		N:P ratio	
		Initial	Final	Initial	Final
Ulva rotundata	1	10.0	13.9	57.4	60.9
	2	27.6	35.9	33.4	38.4
	3	24.9	37.8	34.3	26.0
Enteromorpha intestinalis	1	7.80	12.1	33.5	51.3
	2	7.69	15.8	24.6	34.1
	3	8.08	13.6	20.0	28.6
Gracilaria gracilis	1	9.79	14.3	28.6	47.1
	2	13.4	22.0	38.4	28.5
	3	8.27	16.2	26.2	30.4

the stages, suggesting that algae at the last stage were also strongly N limited.

Discussion

In the previous study (Martínez-Aragón et al. 2002), the same species biofiltered nearly all the phosphate from a sea bass cultivation tank at low flow rates and therefore the integrated system achieved clean effluent discharges. The present study demonstrated that ammonium in effluents released may be also strongly reduced by algal biofiltration at these flow rates, corresponding to ammonium fluxes between 0.82 and 3.26 μmol N L^{-1} h^{-1}. The kinetics of ammonium uptake under the culture conditions for the biofiltration experiments showed that kinetic parameters were within the range of a large data set of values published for various macroalgae (Hein et al. 1995; Pedersen and Borum 1997).

Different studies have pointed out that species of the *Ulva* and *Gracilaria* are ideal candidates for the development of waste water biofiltering integrated polycultures. For instance, Jiménez del Río et al. (1996) designed a system in which *Ulva rigida* stripped with high efficiency (more than 90% at 2 volumes d^{-1} water flow) the dissolved inorganic nitrogen (DIN) in the waste water effluents from a gilthead seabream (*Sparus aurata*) cultivation tank. The removal efficiency decreased when flow rates were increased up to 12 volumes d^{-1}. This system rendered similar growth rates that were obtained in the present study for *U. rotundata*. Recently, in an integrated treatment of a shrimp effluent with oyster and macroalgae, Jones et al. (2001) found that *G. edulis* reduced more than 95% the ammonium concentration in two hours. In addition, the ammonium uptake rate at the NH_4^+ concentration saturating velocity was similar to the value reported in our study. Other efficient systems for DIN using fish pond effluents have been designed under flow rates up to 16 volumes d^{-1}

(Neori et al. 1991). These experiments suggest that the biofiltering efficiency in our system can be optimised, so that greater flow rates should be tested. In fact, the significant increase of the integrated ammonium uptake rates (Figure 3) and the tissue N (Table 4) with the water flow suggests that designs can be substantially improved. In particular, if the flow rate is not adequate for algal growth, eventually there will be more organic N decaying than being produced. In fact, the three-stage design showed that the ammonium concentration entering the second and/or the third stages were insufficient to increase the N biomass in the cultures (Table 7). Further experiments using different species in each stage, when one species alone is unable to reduce the DIN concentration, or under greater flow rates should be tested.

Other studies at a larger scale have also shown that ammonium can be biofiltered in integrated fish-macroalgae cultivation systems, establishing environmental and economic benefits. Buschmann et al. (1996) reported that *G. chilensis* was able to remove 50% of ammonium in winter, increasing up to 95% in spring in a salmon-*Gracilaria* cultivation system, even when the authors pointed out that their strategy did not maximise the biofiltering efficiency. Troell et al. (1997) estimated that, extrapolating to a large scale, *G. chilensis* co-cultivated with salmon had the potential to remove at least 5% DIN released from the fish farm. Neori et al. (1998) reported that *U. lactuca* and *G. conferta* removed about 34% total ammonium supplied in a complex integrated culture.

Algal preincubation under nutrient deprivation affected ammonium biofiltration in *E. intestinalis* and *G. gracilis* (Table 2, Figure 3). The initial ammonium concentration in the starved *E. intestinalis* culture was lower than in unstarved ones (Table 3), and this may affect uptake rates. In fact, final concentrations were lower when algae were previously maintained under nutrient deprivation. In contrast, a moderate N-deprivation period enhanced the ability for ammonium biofiltration in *G. gracilis*. In contrast to green algae, red algae (e.g. *Gracilaria*) may have additional N reservoirs such as phycobiliproteins (Vergara et al. 1995) as well as more dynamic C reserves (specially floridean starch) to facilitate N-assimilation. Thus, following a N-deprivation period, the capacity to take up, accumulate and store N in organic compounds is greater in *G. gracilis* than in the two chlorophytes.

Water flow and the tissue C:N and N:P ratios may control the differences in biofiltering efficiencies. The C:N ratio generally increased slightly during the experiments and this may explain the high efficiencies for ammonium. However, except for the last cultures in the three-stage experiment, values were within the range of the mean C:N values reported for these species (Hernández et al. 1997; Andría et al. (1999, 2001)), which suggests that N was not limiting growth. In addition, although the biomass N content generally decreased during the experiments, final values were greater than the mean 2% of critical tissue N concentrations reported for different macroalgae: 2.2% in *U. lactuca* (Pedersen and Borum 1997), 2.5% in *E. intestinalis* (Björnsäter and Wheeler 1990), 2% in *G. tikvahiae* (Hanisak 1983), which suggest that macroalgae were growing at maximum rates. Only when ammonium was supplied at a low water flow (0.5 volumes d^{-1}) the algae may become N deficient. However, the high N:P ratio and the decrease in tissue P during the experiments indicated that P was the main limiting nutrient (Duarte 1992; Martínez-Aragón et al. 2002).

The ammonium stripped in the biofiltering system rendered an increase in algal N biomass, with gains correlated to the algal growth rate (Figure 4). However, the three-stage design indicated that marked losses in tissue N were observed due to the low inflowing ammonium concentration in the second and third stages. This low inflow decreased algal growth rates (Martínez-Aragón et al. 2002) and caused partial deterioration of tissues and ammonium excretion, as shown by the increase in ammonium outflowing the last stage in *G. gracilis* (Table 6). As a result, losses in N biomass were observed (Table 7). In this experiment, the low tissue N measured after cultivation (lower than the critical quotas for N; see above), specially in the second and third stages, are consequence of the low nutrient input, algal demand and biomass N dilution due to growth. The increase in C:N, and the decrease in N:P ratios as water circulated though the different stages suggests that N limitation in the last culture was likely.

Integrated mariculture represents a promising solution to reduce the N loading from wastes of intensive aquaculture practises and hence adverse impacts on the environment (Neori and Shpigel 1999; Naylor et al. 2000). The present study pointed out the ability of three native macroalgae to diminish substantially the ammonium concentration in effluents from a sea bass cultivation tank and thus improve the water quality. Preliminary results on a scaled up system suggest that it is feasible to design more ecologically sound

practices for sustainable management of aquaculture in southern Spain.

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