Journal of Applied Phycology **14:** 365–374, 2002.

Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 1. Phosphate

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Received 6 November 2001; accepted in revised form 15 August 2002

Key words: Enteromorpha, *Gracilaria*, Integrated aquaculture, Phosphorus, *Ulva*

Abstract

The potential of three estuarine macroalgae (*Ulva rotundata*, *Enteromorpa intestinalis* and *Gracilaria gracilis*) as biofilters for phosphate in effluents of a sea bass (*Dicentrarchus labrax*) cultivation tank was studied. These seaweeds thrive in Cádiz Bay and were also selected because of their economic potential, so that environmental and economic advantages may be achieved by future integrated aquaculture practices in the local fish farms. The study was designed to investigate the functioning of P nutrition of the selected species. Maximum velocity of phosphate uptake (2.86 μmol PO_4 g^{-1} dry wt h^{-1}) was found in *U. rotundata*. This species also showed the highest affinity for this nutrient. At low flow rates (< 2 volumes d^{-1}), the three species efficiently filtered the phosphate dissolved in the waste water, with a minimum efficiency of 60.7% in *U. rotundata*. Net phosphate uptake rate was significantly affected by the water flow, being greatest at the highest rate assayed (2 volumes d^{-1}). The marked decrease in tissue P shown by the three species during a flow-through experiment suggested that growth was P limited. However, due to the increase in biomass, total P biomass increased in the cultures. A significant correlation was found between growth rates and the net P biomass gained in the cultures. A three-stage design under low water flow (0.5 volumes d^{-1}) showed that the highest growth rates (up to 0.14 d^{-1}) and integrated phosphate uptake rates (up to 5.8 μmol PO_4^{3-} g^{-1} dry wt d^{-1}) were found in *E. intestinalis* in the first stage, with decreasing rates in the following ones. As a result, phosphate become limiting and low increments or even losses of total P biomass in these stages were found suggesting that phosphate was excreted from the algae. The results show the potential ability of the three species to reduce substantially, at low water flow, the phosphate concentration in waste waters from a *D. labrax* cultivation tank, and thus the quality of effluents from intensive aquaculture practices.

Introduction

Intensive fish farming activities may cause several ecological impacts, as habitat modification, wild seedstock collection and coastal eutrophication (reviewed by Naylor et al. (2000)). Untreated waste water laden with uneaten food and excretory products from fish is one of the main causes of nutrient pollution in shallow and/or confined coastal ecosystems, especially if intensive aquaculture practices are concentrated (Iwama 1991). To achieve a more sustainable farming, the promotion of environmentally sound aquaculture practices is necessary. The Bangkok Declaration and Strategy for Aquaculture Development Beyond 2000, emanated from the Conference on Aquaculture in the Third Millennium stated that integrated aquaculture can add value to the current use of on-farm resources and recommended the development of sustainable practices to improve environmental performance, using aquatic plants for nutrient stripping (see also Naylor et al. (2000)).

The use of seaweeds as purification agents for domestic sewage and agriculture effluents has been proposed before (e.g. Kindig and Littler (1980) and Chan et al. (1982)). Various studies have shown that several species have high biofiltering capacities and thus can contribute to an efficient removal of dissolved P and N wastes from intensive fishpond systems (Haglund and Pedersén 1993; Krom et al. 1995; Neori et al. 1996). In addition, the development of such integrated farming (polyculture) systems can increase the economic output of the activity (Troell et al. 1997). Most of the studies are focused on dissolved N removal (especially ammonium) (e.g. Neori et al. (1996)). In contrast, few studies have addressed the efficiency of P biofiltration. For instance, the integrated farming of *Gracilaria chilensis* and salmon cages in Chile had the potential to remove at least 27% of released phosphate from the fish farm (Troell et al. 1997).

According to Chopin et al. (2001), a main issue in the effective implementation of a biofiltering system is their optimal functioning, which requires in-depth understanding of the physiology and nutrition. The optimization of the overall efficiency of a cultivation system at low scale (laboratory study) will require a compromise between the water flow, the nutrient uptake and the biomass production.

In Cádiz Bay, an enclosed shallow ecosystem of high environmental value, intensive and semi-intensive aquaculture is developing into a strong industry. In recent years, stringent legislative controls specify the maximum allowed nutrient concentrations in the effluent discharges and thus the farming industry is addressing the reduction of nutrients in the effluent outputs to the environment. As part of a current research project, the use of native benthic macroalgae as potential biofilters of the dissolved nutrient effluents from fish farming activities is being investigated. The species were selected as they are also profitable. For instance, they can be used as animal food (*Ulva*: Arieli et al. (1993) and Neori et al. (1998)), agar source (*Gracilaria*: Troell et al. (1997)) or human food (*Enteromorpha*: Chapman and Chapman (1980)).

The aim of the present study is to assess the capability of three macroalgae (*Ulva rotundata*, *Enteromorpha intestinalis* and *Gracilaria gracilis*) as potential biofilters of dissolved wastes from an experimental tank cultivation of sea bass. The laboratory study consisted of algal cultures supplied with low flow rates (< 2 volumes d^{-1}) in the hope of attaining high phosphate uptake efficiencies. A similar study on ammonium, the main dissolved inorganic N source, is reported in a second paper (Hernández et al. 2002). The biofiltering and economic potential of these species may thus encourage future polyculture systems to be adopted by the local farmers as an environmentally friendly way of recycling the waste waters from intensive aquaculture practices.

Materials and methods

Algal material and preculture conditions

The seaweeds were collected from two locations in Cádiz Bay. *Gracilaria gracilis* (Stackhouse) Steentoff, L.M. Irvine & Farnham was harvested from tidal channels in Los Toruños, a salt marsh located in the vicinity of the laboratory. *Ulva rotundata* Bliding and *Enteromorpha intestinalis* (L.) Ness were collected from pools at an intensive fish farm (ACUINOVA S.L., near San Fernando, Cádiz). The material was carried to the laboratory under humid and cool conditions in an ice-chest. In the laboratory, the algae were precultured for one week under continuous flow of seawater pumped from a natural underground well (mean nutrient concentrations: 0.8 μM PO_4^{3-}, 34 μM NH_4^+, and negligible NO_3^-). The precultures consisted of aerated, fibreglass tanks of 235 L (0.6 m^2 surface area) illuminated with white light fluorescent lamps (TFC FL30SD) at 180 μmol photon m^{-2} s^{-1}, in a 12:12 LD photoperiod. During the preculture period the algae remained healthy and, if necessary, depigmented areas or visible epiphytes were carefully removed. From these stocks, specimens were selected for the experiments.

Kinetics of phosphate uptake

To assay the kinetics of PO_4^{3-} uptake, 5 g fresh weight (fw) algae were passed to 1 L volumetric flasks supplied with a continuous flow (2 volumes d^{-1}) of waste water from a sea bass cultivation tank of 8.5 m^3 (3 kg fish m^{-3}). The source of seawater was the same as used during the preculture period. However, the excretory products from the fish and the uneaten food increased phosphate by about 50%, although concentrations up to 5 μM were measured. The conditions in the cultures were 18 °C and 180 μmol photon m^{-2} s^{-1}. After 24 h the flow was stopped and water samples were taken at 0, 1, 2, 3, 6

and 7 h to follow PO_4^{3-} disappearance. The experiments were run in triplicate. A blank (culture flask without algae) was also sampled. The nutrient uptake rates were determined as $(C_t - C_{t+1})V/(B\ \Delta t)$, where C represents the phosphate concentration (μM); V represents the volume (L); B represents the algal biomass; and Δt the time elapsed between t and t + 1. Uptake rates were expressed as μmol PO_4^{3-} g dry wt^{-1} h^{-1}. Kinetic uptake parameters (V_{max}, the maximum uptake rate and K_s, the half saturation value) were calculated from the Michaelis-Menten equation.

Experimental set-up

A flow-through design was developed to test the biofiltering capacity of the species at three flow rates (0.5, 1, and 2; volumes d^{-1}). The design was run with and without a previous step of algal maintenance under nutrient deprivation (6 days in oligotrophic seawater collected offshore). Waste water was pumped from a container (50 L) from the fish tank into a 2 -L volumetric flask containing 2 g fw of alga. The flasks were aerated and illuminated with 240 μmol photon m^{-2} s^{-1}, in a 12:12 LD photoperiod. Water was pumped using a peristaltic pump (Watson Marlow 205S/CA) of 16 channels equipped with Marprene® manifold tubings. Tubes of different diameters were used to culture algae at the three turnover rates. Water samples were collected for phosphate analysis at the inflow and at the outflow of each flask. The experiment was run for one week.

The biofiltering capacity of the species was also tested in a three-stage design. In this case, the peristaltic pump circulated the waste water from the fish cultivation tank to three stages of in/outflow volumetric flasks containing the algae (1 g fw L^{-1}), at a water flow of 0.5 volumes d^{-1}. Seaweeds were previously cultured for 6 days under nutrient deprivation. Light and photoperiod were as above. Again, nutrient concentration was measured at the inflow and outflow of each flask. The whole experiment lasted for 10 days (10, 8 and 6 days for the first, second and third stage respectively, as the second flask had to be complemented with water outflowing the first culture and the third flask with water from the previous one).

After each experiment, algae were weighed to calculate specific growth rates (d^{-1}), assuming exponential growth during the experimental period. Finally, the algae were dried at 60 °C for 3 days. The dried tissue was ground and stored in vials for further tissue P content analysis.

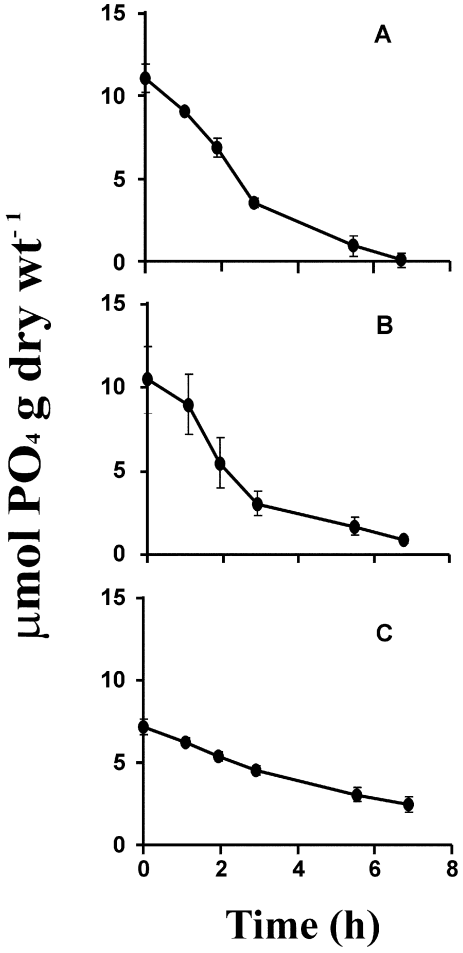

Figure 1. Kinetics of phosphate disappearance from the medium. A) *Ulva rotundata*; B) *Enteromorpha intestinalis*; C) *Gracilaria gracilis*. Data are mean ± SD (n = 3).

Table 1. Kinetic parameters of phosphate uptake of 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 constant, μM) and V_{max}/K_s, an estimator of the uptake affinity. Data are mean ± SD (n = 3)

Species	V_{max}	K_s	V_{max}/K_s
Ulva rotundata	2.86 ± 1.01	0.45 ± 0.83	6.36
Enteromorpha intestinalis	2.64 ± 0.89	0.63 ± 0.39	4.19
Gracilaria gracilis	1.25 ± 0.39	1.64 ± 0.18	0.76

The net rate of phosphate uptake for a given interval of time (i, i + 1) was calculated from a modification of the formulae given by Carmona et al. (1996):

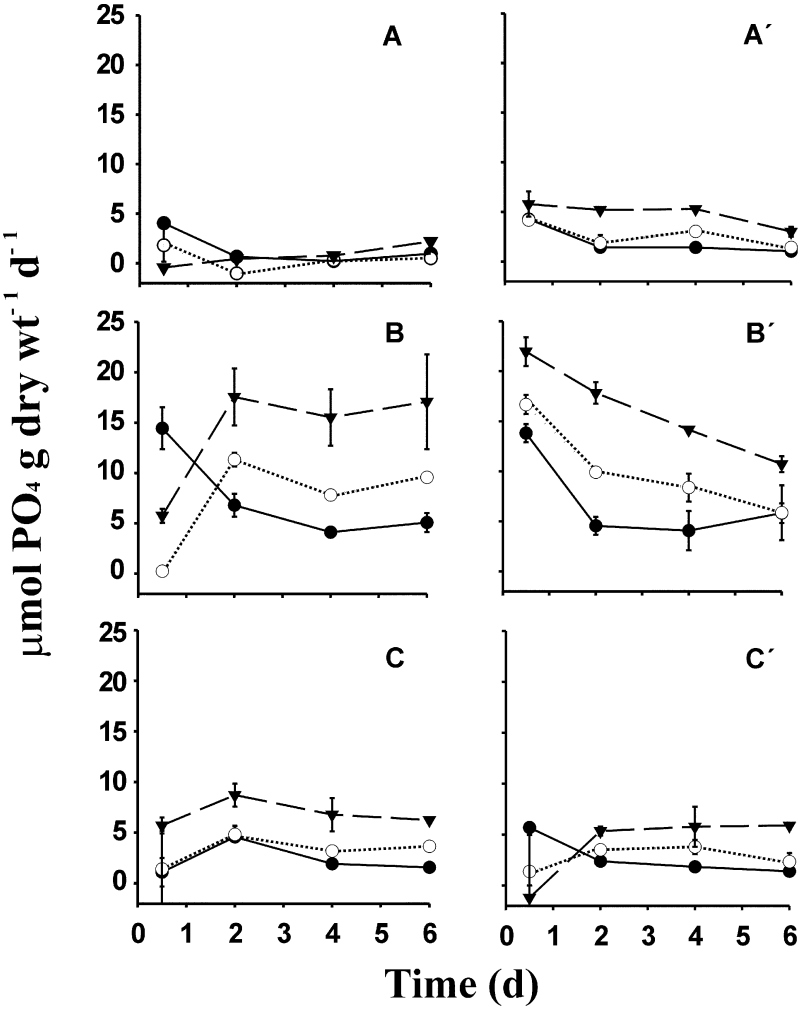

Figure 2. Net phosphate uptake rates versus time at water flows of 0.5 (●), 1 (○), and 2 (▼) volumes d^{-1} without a previous step of algae maintenance under nutrient deprivation (normal caps) or with such step of 6 days in oligotrophic seawater (prime caps). A–A′) *Ulva rotundata*; B–B′) *Enteromorpha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

$$\mu molPO_4^{3-}\,g^{-1}\text{dry}\quad \text{wt}d^{-1} = (Cout_i V + Q\Delta t Cin_{(i,i+1)} - Q\Delta t Cout_{(i,i+1)} - Cout_{i+1}V)/B\Delta t$$

where C*in* and C*out* = mean inflow and outflow phosphate concentration (μM) during the time interval (i, i + 1), respectively; V = volume (L); Q = flow of seawater (L d^{-1}); B = algal biomass (g dry wt) during the time interval (Δt, days) considered. The four terms in the equation represent the existing phosphate in the cultures at time *i*, plus the inflowing phosphate concentration, minus the outflowing concentration, minus the remaining phosphate in the cultures at time i + 1. This equation is preferred over standard chemostat formulations as nutrient concentration in the inflow may not be in steady state.

For the calculations, the following dry wt: fresh wt ratios were estimated (n = 15): 0.161 (*U. rotundata*), 0.100 (*E. intestinalis*), and 0.137 (*G. gracilis*).

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.

Chemical analyses

Phosphate was determined as soluble reactive P, according to Murphy and Riley (1962). Water samples were previously filtered through Whatman GF-F fil-

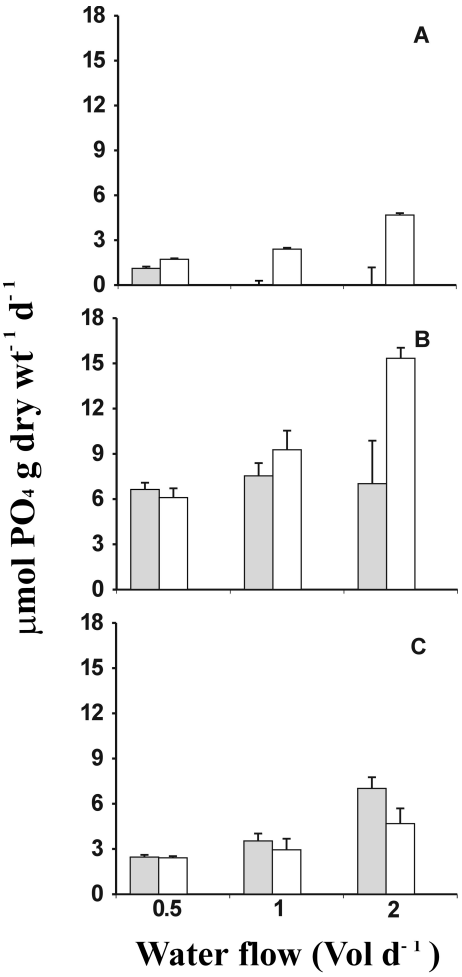

Figure 3. Integrated phosphate uptake rates at different flow rates. Full bars represent rates measured in unstarved cultures. Empty bars represent rates under a previous step of 6 days in oligotrophic seawater. A) *Ulva rotundata*; B) *Enteromorpha intestinalis*; C) *Gracilaria gracilis*. Data are mean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

ters. Tissue P content was analysed by acid digestion in triplicate samples (Sommer and Nelson 1972).

Statistics

The overall effects of the previous step of macroalgal maintenance under nutrient deprivation and water flow rates on integrated rates of P uptake were analysed by a two-way ANOVA (model I). Multiple post hoc comparisons among means were tested by the Tukey test (Zar 1984). The relation between macroalgal growth rates and the increase in net P biomass during the flow-through design was analyzed 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 phosphate dissolved in the waste water from the fish tank (Figure 1). After 7 h of incubation, most of the phosphate was taken up by the macroalgae, as nutrient concentration remained constant in the control cultures (data not shown). Removal efficiency was highest in *U. rotundata* (99.6%) and lowest in *G. gracilis* (62.2%).

The maximum uptake rate of phosphate occurred in *U. rotundata*, slightly greater than that calculated for *E. intestinalis*, while *G. gracilis* showed the lowest V_{max} (Table 1). The latter species also showed the lowest affinity (indicated by K_s) The efficiency of phosphate uptake, defined by the ratio V_{max}/K_s was also lowest in *G. gracilis* (Table 1), while the highest values were again estimated for *U. rotundata*.

Net phosphate uptake rates were generally affected by the seawater flow, being greatest under 2 volumes

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 integrate rates of phosphate uptake of three macroalgal species. DF: Degrees of freedom; MS: Mean square

Species	Factor	DF	MS	F-value	p
Ulva rotundata	Preincubation	1	15.1	104	< 0.01
	Water flow	2	3.13	21.6	< 0.01
	Interaction	2	2.59	18	< 0.01
Enteromorpha intestinalis	Preincubation	1	0.82	0.24	0.64
	Water flow	2	98.8	28.5	< 0.01
	Interaction	2	2.0	0.57	0.58
Gracilaria gracilis	Preincubation	1	1.04	2.73	0.13
	Water flow	2	20.0	52.7	< 0.01
	Interaction	2	0.38	1.01	0.40

Table 3. Phosphate biofiltration efficiency at the end of the flow-through experiment (day 7) and growth rate of the three macroalgal species under different flow rates. Experimental designs were run with (starved cultures) and without (unstarved cultures) a previous step of plant maintenance under nutrient deprivation. Data are mean of three replicates ± SD

Species	Water flow (volumes d^{-1})	Unstarved cultures		Starved cultures	
		PO_4 filtration efficiency (%)	Growth rate (d^{-1})	PO_4 filtration efficiency (%)	Growth rate (d^{-1})
Ulva rotundata	0.5	89.4	0.19 ± 0.03	89.7	0.12 ± 0.02
	1	60.7	0.20 ± 0.06	76.9	0.12 ± 0.02
	2	96.2	0.19 ± 0.02	69.2	0.13 ± 0.03
Enteromorpha intestinalis	0.5	99.2	0.10 ± 0.04	91.5	0.08 ± 0.03
	1	99.6	0.10 ± 0.01	85.3	0.10 ± 0.01
	2	91.6	0.15 ± 0.01	85.4	0.17 ± 0.01
Gracilaria gracilis	0.5	93.9	0.09 ± 0.01	71.4	0.06 ± 0.01
	1	98.0	0.08 ± 0.02	81.0	0.08 ± 0.01
	2	93.9	0.09 ± 0.01	78.6	0.07 ± 0.01

d^{-1}, and lowest at 0.5 volumes d^{-1} (Figure 2). The lowest uptake rates were observed in *U. rotundata*, especially in unstarved plants. The integrated rates of phosphate uptake increased significantly with the seawater flow (Figure 3; ANOVA results in Table 2). In addition, integrated phosphate uptake rates of *U. rotundata* were significantly higher in starved cultures, whereas there was no significant effect of previous maintenance with or without P deprivation in *G. gracilis* and *E. intestinalis* (Table 2).

Table 3 shows the phosphate biofiltration efficiency and the grow rate of the three species when water was circulated at flow rates of 0.5, 1 and 2 volumes d^{-1} respectively. Growth rates were usually greater in unstarved cultures. Despite the low net phosphate uptake rates, the highest growth rates were found in unstarved *U. rotundata* cultures at all exchange rates. Lowest growth rates were always found in *G. gracilis*, especially in starved cultures.

Phosphate was biofiltered at a high percentage in all cultures, with values always above 60% of PO_4^{3-} reduction at the end of the experiments. Uptake efficiencies were usually greater in unstarved cultures, where higher growth rates were found (Table 3). In some cases (e.g., unstarved cultures of *E. intestinalis*), phosphate was almost completely extinguished, leaving negligible concentrations in the outflow water at the end of the experiment.

Changes in tissue P content are shown in Table 4. Tissue P content at the beginning of the experiment diminished when the algae were preincubated under nutrient deprivation. After the experiment, and despite the high production of macroalgal tissue, all species under all flow rates showed a marked decrease in tissue P, with losses as high as 72%. Losses were particularly high in unstarved cultures of *U. rotundata*. However, the net P biomass increased in the cultures due to biomass production (Table 3). The P biomass produced was similar to the total phosphate input to the system (data not shown). Moreover, pooled data from the three species showed a significant correlation between growth rates and net P bio-

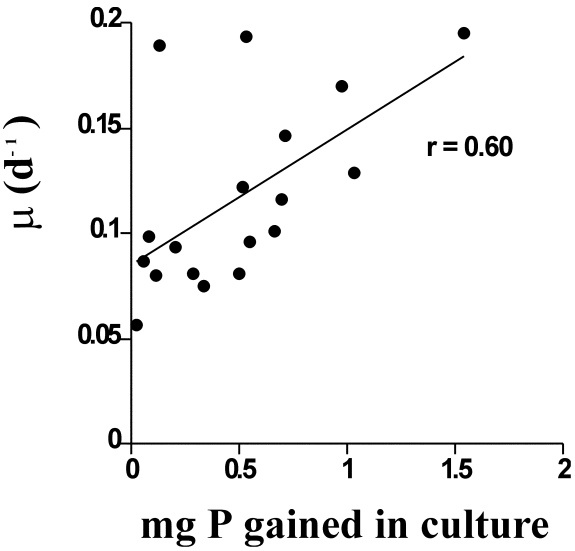

Figure 4. Correlation between growth rate and net P biomass gained in cultures during the flow-through experiments (r = 0.59, p = 0.007).

Table 4. Macroalgal tissue P concentrations at the initial and at the end of the experiments, percentage loss in tissue P and total P biomass gained during the flow-though experiments performed under different flow rates. Experimental designs were run with (starved cultures) and without (unstarved cultures) a previous step of algal maintenance under nutrient deprivation. Data are mean of three replicates ± SD

Species	Water flow (volumes d^{-1})	Unstarved cultures				Starved cultures			
		Tissue P (mg g^{-1} dry wt)		% loss per dry wt	mg P gained in culture	Tissue P (mg g^{-1} dry wt)		% loss per dry wt	mg P gained in culture
		Initial	Final			Initial	Final		
Ulva rotundata	0.5	1.68 ± 0.12	0.48 ± 0.02	71.6	0.130	1.12 ± 0.03	0.68 ± 0.05	38.9	0.514
	1	1.68 ± 0.12	0.80 ± 0.10	52.5	1.54	1.12 ± 0.03	0.79 ± 0.01	29.4	0.697
	2	1.68 ± 0.12	0.87 ± 0.12	48.5	0.532	1.12 ± 0.03	1.05 ± 0.28	5.8	1.03
Enteromorpha intestinalis	0.5	1.50 ± 0.60	1.20 ± 0.44	20.0	0.662	1.29 ± 0.05	1.19 ± 0.16	8.5	0.500
	1	1.50 ± 0.60	0.81 ± 0.06	45.8	0.079	1.29 ± 0.05	0.91 ± 0.08	29.8	0.547
	2	1.50 ± 0.60	1.07 ± 0.06	28.8	0.710	1.29 ± 0.05	1.11 ± 0.09	14.0	0.976
Gracilaria gracilis	0.5	2.93 ± 0.25	2.03 ± 0.04	30.7	0.205	2.65 ± 0.15	1.68 ± 0.12	36.6	0.025
	1	2.93 ± 0.25	2.35 ± 0.03	19.8	0.288	2.65 ± 0.15	1.90 ± 0.23	28.2	0.115
	2	2.93 ± 0.25	1.68 ± 0.03	42.7	0.059	2.65 ± 0.15	1.93 ± 0.16	27.0	0.340

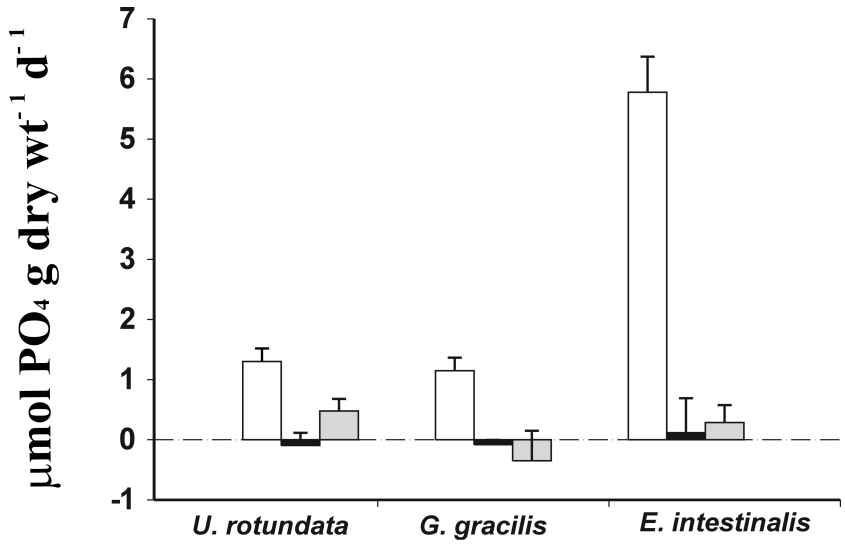

Figure 5. Integrated phosphate uptake rates of the species in the three-stage design. White bars: first stage. Black bars: second stage. Grey bars: third stage. Data are mean ± SD (n = 2, 3).

mass gained in the cultures (r = 0.59, p = 0.007; Figure 4).

Figure 5 shows the integrated rates of phosphate uptake in the three-stage design. As in the previous experiment, the highest uptake rate (first culture) was measured in *E. intestinalis*. This culture reduced drastically the phosphate concentration in the water, hence, low or negligible rates were measured in the following stages. In some occasions negative rates were observed (e.g. last culture of *G. gracilis*), indicating that some phosphate was excreted by the algae.

Table 5 shows the phosphate biofiltering efficiency and growth rate of the macroalgae in the three-stage design. Due to the greater uptake rates, the highest growth rates were always measured in the first stage,

Table 5. Mean phosphate concentration at the inflow and outflow of the three-stage design at water flow of 0.5 volumes d^{-1}, phosphate filtration efficiency at the end of the experiments and macroalgal growth rate. The accumulated phosphate biofiltration estimated at the outflow of each stage is given in brackets. Data are mean of three replicates ± SD. Some values for efficiency are negative, because mean outflow phosphate concentration was higher than inflow concentration

Species	Biofiltration stage	PO_4 (μM)		PO_4 filtration efficiency (%)	Growth rate (d^{-1})
		Inflow	Outflow		
Ulva rotundata	1	0.96 ± 0.49	0.24 ± 0.15	75.0	0.06 ± 0.008
	2	0.24 ± 0.15	0.23 ± 0.21	4.2 (76.1)	0.04 ± 0.009
	3	0.23 ± 0.21	0.14 ± 0.14	39.1 (85.8)	0.02 ± 0.005
Enteromorpha intestinalis	1	2.2 ± 0.2	0.36 ± 0.31	83.6	0.14 ± 0.04
	2	0.36 ± 0.31	0.51 ± 0.26	–39.1 (76.9)	0.08 ± 0.03
	3	0.51 ± 0.26	0.54 ± 0.25	–5.9 (75.4)	0.11 ± 0.004
Gracilaria gracilis	1	0.66 ± 0.10	0.14 ± 0.183	78.6	0.06 ± 0.005
	2	0.14 ± 0.183	0.10 ± 0.08	28.6 (84.8)	0.04 ± 0.001
	3	0.10 ± 0.08	0.16 ± 0.16	–60.0 (75.6)	0.04 ± 0.005

Table 6. Macroalgal tissue P concentration at beginning and end of the experiments, percentage loss in tissue P content and total P biomass gained during the three-stage design. Data are mean of three replicates ± SD

Species	Biofiltration stage	Tissue P (mg g^{-1} dry wt)		% loss per dry wt	mg P gained in culture
		Initial	Final		
Ulva rotundata	1	1.12 ± 0.08	0.68 ± 0.06	56.5	0.239
	2	0.71 ± 0.09	0.46 ± 0.05	34.9	–0.024
	3	0.82 ± 0.08	0.63 ± 0.04	23.0	0.009
Enteromorpha intestinalis	1	2.65 ± 0.08	0.98 ± 0.04	57.5	0.351
	2	1.44 ± 0.10	0.94 ± 0.11	58.9	–0.072
	3	2.88 ± 0.29	1.40 ± 0.11	46.9	0.107
Gracilaria gracilis	1	2.13 ± 0.19	0.91 ± 0.03	63.0	–0.010
	2	2.56 ± 0.15	1.05 ± 0.07	34.7	0.062
	3	2.91 ± 0.35	1.54 ± 0.39	51.5	–1.0

with decreasing rates in the following ones. Phosphate was biofiltered at a high efficiency, with similar percentages among the three species. In *E. intestinalis* and *G. gracilis*, phosphate concentration increased as water outflowed the first culture and circulated through the following stages (as shown also by the lower phosphate removal efficiency; Table 5), suggesting again that phosphate was excreted from the algae.

Changes in tissue P content are shown in Table 6. The three species showed a marked decrease in tissue P after the three-stage experiment, with tissue P losses up to 63%. The low water flow (0.5 volumes d^{-1}) and the previous starvation of the algae led to low increments in the net P biomass in the first stage, with even negative values for *G. gracilis*. The high reduction of phosphate observed led to low concentrations in the following stages, which subsequently caused low increments (or even losses) of P biomass. In fact, thalli in some of the following stages began to disintegrate at the end of the experiment.

Discussion

The development of polyculture systems represents a promising solution by integrating the cultivation of macroalgae into finfish culture (e.g. Troell et al. (1997) and Neori and Shpigel (1999)). Our results show that, at low water flow corresponding to phosphate fluxes between 0.026 and 0.102 mol P L^{-1} h^{-1}, *U. rotundata*, *E. intestinalis* and *G. gracilis* stripped nearly all the phosphate from fishpond effluents, thereby greatly improving water quality. *U. rotundata* showed the highest V_{max} of phosphate uptake but the lowest net uptake rate in the flow-through design (Figure 2). This apparent contradiction is due to the fact that kinetic assays were performed at higher

phosphate concentrations than those encountered in the flow-through experiment. It also remarks the difference between the surge uptake response (i.e. hours, phosphate-pulsed, closed system) and the internal controlled uptake (days, open system) in this species (Pedersen 1994). Other biofiltration systems using *Ulva* as potential phosphate biofilter were designed under flow rates up to 8 volumes d^{-1} (Vandermeulen and Gordin 1990) and rendered similar growth rates as were obtained in this study for *U. rotundata*. These results indicate that our system can be optimised to higher flow rates. In particular, if the water flow is not adequate for algal growth, there may be more organic P decaying than being produced. In fact, the three-stage design showed that the low phosphate concentration inflowing the second and/or third stages were insufficient to increase the amount of tissue P in the cultures. We acknowledge that in order to scale up and improve the biofiltration capacity of the system, future experiments should test different species in each stage under higher flow rates.

There are apparently no previous studies testing the ability of *Enteromorpha* as biofilter. However, several studies have shown that phosphate can be biofiltered efficiently in integrated fish-*Ulva* and/or *Gracilaria* cultivation systems. Buschmann et al. (1996) reported that *Gracilaria chilensis* removed 32% of the phosphate in an integrated salmon-*Gracilaria* cultivation system, even when the authors pointed out that the cultivation strategy followed did not maximise the biofiltration efficiency of the algae. Troell et al. (1997) estimated that, extrapolating to a large scale, *G. chilensis* cultivation would be capable of removing 27% of phosphate in effluents from salmon cages. Neori et al. (1998) reported that *U. lactuca* and *Gracilaria conferta* removed less than 25% of the phosphate added in a complex integrated system, however they found filtration efficiencies up to 84,8% over 24 h. Recently, Jones et al. (2001) showed that *Gracilaria edulis* reduced the phosphate concentration from 3.3 to 0.16 μM (95%) in shrimp effluents.

Other studies have shown low removal efficiency of phosphate by biofiltration (DeBoer et al. 1978; Neori et al. 1996). In these cases, factors as water flow and the tissue N:P ratio may control differences in biofiltering efficiency (Chopin et al. 2001). In the present study, tissue N:P ratios greater than mean N:P values reported for macroalgae (Duarte 1992; Hernández et al. 1997), were found (Hernández et al. 2002), which may partly explain the high biofiltering efficiencies found in these species.

Most of the phosphate stripped in the biofiltering system was accounted for by subsequent gains in algal biomass. However, although the total P biomass produced in the cultures increased during the experiments, with gains correlated to the algal growth rate (Figure 4), tissue P decreased markedly. That suggests that P supply was inadequate to sustain maximum growth, indicating P limitation even when the macroalgae were not previously incubated under phosphate deprivation. This was shown clearly in the three-stage design, where the low phosphate concentration inflowing the second flask and the low tissue P caused partial deterioration of tissues and phosphate excretion that in some species (e.g. *E. intestinalis*) led to an increase in the net P biomass gained in the following stages.

Variations in tissue P reflect inherent species-specific differences in the ability to sequester nutrients. Nutrient levels reached after cultivation are the consequences of the nutrient input, algal demand and biomass dilution due to growth. The tissue P measured after the biofiltering experiments suggests that values were below critical concentrations (the tissue P needed to support maximum growth). According to the P critical quota reported for *Ulva* and other macroalgae (Pedersen 1993; Delgado et al. 1996; Lyngby et al. 1999), the tissue P content in *U. rotundata* and *E. intestinalis*, and to a lesser extent in *G. gracilis* strongly suggested that seaweeds were P-limited, in particular at the lowest water flow and when algae were previously incubated under P deprivation. In these conditions the algae were growing below maximum rates. The tissue N:P ratios (Hernández et al. 2002) strongly supported our hypothesis.

Not only does integrated seaweed production benefit coastal ecosystems by reducing P loading, and hence the risk of eutrophication. The three macroalgae tested also represent a marketable product and can increase the economic output of a fish farm. The present study was based on small scale experiments and has been complemented with the biofiltering efficiencies for ammonium, the inorganic-N form in the sea bass waste water (Hernández et al. 2002). The possibility of scaling up the implementation of this system to a mesoscale, under larger time intervals, is now being investigated in a fish farm, and preliminary results suggest that it is feasible to design promising, integrated system approaches, to the management of aquaculture in southern Spain.

Acknowledgements

The authors thank M. A. Fernández-Engo for technical assistance and S. Tirado and personnel of the Marine Culture Laboratory of the University of Cádiz for technical support. The authors also thank E. J. Malta for critical review of an earlier version of the manuscript. The present research is a contribution to project 1FD1496 of the Spanish Interministerial Commission of Science and Technology (CICYT) and the European Commission.

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