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Integrated outdoor culture of two estuarine macroalgae as biofilters for dissolved nutrients from *Sparus aurata* waste waters

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Abstract

An integrated outdoor cultivation of two macroalgal species: *Ulva rotundata* (Chlorophyta) and *Gracilariopsis longissima* (Rhodophyta) was designed. The macroalgae were cultured in effluents from an intensive marine culture (growout phase) of gilthead seabream *Sparus aurata*. The biomass evolution of the algal tanks followed a logistic curve, where the approach to the maximum stocking density of seaweeds was governed by thalli self-shading, as nutrient limitation in the cultivation tank was unlikely. The maximum stocking density of the system was approximately 27.8 g *U. rotundata* L^{-1} (16.7 Kg m^{-2}) and 11.9 g *G. longissima* L^{-1} (7.12 Kg m^{-2}). Yield was more than 3 times higher in *U. rotundata* than in *G. longissima*. Overall, *U. rotundata* removed a greater percentage of phosphate (8.9%) and total dissolved inorganic nitrogen (54%) flowing into the algal tanks than *G. longissima*. The latter species biofiltered approximately 3.2% of phosphate and 17% of the total dissolved inorganic nitrogen input. However, mean nutrient uptake rates on wet weight basis were usually higher in *G. longissima* than in *U. rotundata*. The production of total oxidised nitrogen in the algal tanks, considered as being the nitrification rate occurring on the algal fronds by nitrifying bacteria, was less than half of the ammonium uptake by the macroalgae, suggesting that seaweeds competed efficiently for ammonia against the nitrifyers. The biofiltration during a diel cycle showed that mean phosphate biofiltration was lower than 4.5% in the two species whereas ammonium was biofiltered efficiently (up to 67%), especially in *U. rotundata*. The metal and heavy metal content in the algal tissue at the end of the monitoring period suggested no metal contamination of tissues so that both macroalgal species could be used in the food industry. The study reveals the value of ecological engineering techniques in reducing the dissolved nutrient content in effluents from the fish farm, with the prospect of a better management practises, based on integrated mariculture designs, being developed by the local farmers.

Abbreviations: ToxN, Total Oxidised Nitrogen; DIN, Dissolved Inorganic Nitrogen

Introduction

Intensive fish farming may cause several environmental problems. Among them, coastal eutrophication has been addressed as one of the main ecological impacts, especially in shallow or confined water bodies (reviewed by Naylor et al., 2000). The untreated wastewater laden with uneaten feed and excretory products from fish may cause severe nutrient pollution, especially if intensive aquaculture practises are geographically concentrated (Chopin et al., 2001). The Bangkok Declaration and Strategy for Aquaculture Development Beyond 2000, 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 practises to improve environmental performance, using aquatic plants as nutrient strippers (NACA/FAO, 2000; Naylor et al., 2000). These integrated practises (polyculture) have shown that waste water nutrients from aquaculture can be used as resources for new products, lessening the nutrient concentration in the effluents and increasing the economic output of the activity (Troell et al., 2003).

For instance, the integrated culture of the gilthead seabream *Sparus aurata* and the chlorophyte *Ulva lactuca* removed most of the ammonia excreted by the fish and oxygenated the water (Neori et al., 1996). The integrated culture of the rhodophyta *Gracilaria chilensis* and salmon cages resulted in a substantial reduction of dissolved ammonium and phosphate released from the fish farm, reducing the risk of eutrophication (Troell et al., 1997). Simulation models for some of these systems have been developed successfully (Ellner et al., 1996). These designs, based on ecological engineering approaches (Mitsch and Jorgensen, 1989), provide a practical solution to major management and environmental problems of land-based mariculture and add revenue to the fish farm industry (Troell et al., 1997, 2003; Tacon and Forster, 2003).

In Cádiz Bay, an enclosed shallow ecosystem belonging to the Cádiz Bay Natural Park, finfish mariculture is developing into a strong industry. In recent years, stringent legislative policies specify the maximum allowed nutrient concentrations in the effluent discharges, and thus, the farming industry is addressing the reduction of nutrients in the effluent. Recent studies (Hernández et al., 2002; Martínez-Aragón et al., 2002) have shown that flow-through cultures of *Ulva rotundata, Enteromorpha intestinalis* and *Gracilariopsis longissima,* three profitable estuarine macroalgae thriving in Cádiz Bay, biofiltered efficiently the dissolved ammonium and phosphate from a sea bass *Dicentrarchus labrax* cultivation tank at a low scale (laboratory study). These studies provided a detailed understanding of the physiology of the species under laboratory systems. The aim of the present work is to scale up to the field the capability of *U. rotundata* and *G. longissima* to biofilter dissolved nutrients from an intensive fish farm industry. To cope with this objective, the possibility of cultivation in mesocosm of the two species under natural conditions of light, temperature and photoperiod was assessed. In addition, the content of some nutritional components and heavy metals in both species was analysed to assess if *U. rotundata* and *G. longissima* could be used as human food in the region. The former alga may be used for human consumption whereas the latter is also an important agar source (Indergaard and Minsaas, 1991). The biofiltering and economic potential of these macroalgae may thus encourage future polyculture systems to be adopted by the local farmers as an environmentally friendly way of recycling the waste waters of intensive aquaculture practises.

Materials and methods

Algal cultures

Ulva rotundata Bliding and *Gracilariopsis longissima* (Stackhouse) Steentoff, L.M. Irvine and Farnham, were collected from intertidal pools at an intensive fish farm (ACUINOVA, 25 km from the laboratory). The latter species was named as *Gracilaria gracilis* in previous studies (Hernández et al., 2002; Martínez-Aragón et al., 2002) due to a lack of definitive identification. However, recent studies based on molecular identification of plastid rcbl DNA assigned our samples as *Gracilariopsis longissima* (Gurgel et al., 2003).

Healthy thalli (500 g of initial stocking biomass per tank) were carefully rinsed to remove the mud and placed in two different outdoor PVC tanks of 600 L (1 m^2 surface) under natural conditions. An additional tank without algae, similar to the above, was used as a control. The sea water was pumped from an earthen pond (mean nutrient concentration: 1.4 μM PO_4^{3-}, 5.6 μM NH_4^+, 3.5 μM NO_3^-; Pérez-Pastor, 2005), filtered by mechanical sand filtration and circulated through an intensive culture (growout phase) of *Sparus aurata* L (5.94 $\pm$ 1.50 tonnes), where the excretory products from the fish and the uneaten feed increased the nutrient concentration significantly. Due to the high nutrient concentration in the effluents from the fish culture, outflow water was previously diluted with sea water from the earthen pond (2.5 volume of sea water per volume of effluent) and pumped again to the macroalgal tanks, where water circulated at a flow rate of 3.5 volumes d^{-1}. Dilution with clean water was monitored from a caudalimeter whereas the flowing rate was carefully determined by a straightforward volumetric measure and controlled by a valve. The system was checked periodically. Water was previously passed through Perlon filters to eliminate solid particles. The fishes were fed from 10 a.m. to 2 a.m. at a feeding rate of 0.021 kg feed kg $fish^{-1}$ d^{-1}. The feed ratio was constant during the study period. The tanks were aerated by air diffusers placed at the bottom of each tank so that sea-

weeds were moving while being kept suspended in the water. The system operated from mid-February 2001 to the beginning of May 2001, when the macroalgae reached maximum biomass due to the limits imposed by the carrying capacity of the system under the culture conditions. At this time, the algal biomass was reduced 50% and samples were immediately collected for metal content analysis. The system continued operating until mid-June just to maintain the cultures but no further significant data were recorded. Temperature, salinity and pH records were measured each sampling day with a 3185 probe equipped with a 3800 water quality logger (YSI, Grant Instruments).

The algae were weighted weekly to determine the increase in biomass and calculate specific growth rates (d^{-1}). The algae were placed in a plastic basket to drain off excess water. Subsamples were dried for three days at 60 °C. The dried tissues were ground and stored in vials for further tissue C, N and P content analyses. Visible epiphytes (*Chaetomorpha* spp. and *Enteromorpha* spp.) were carefully removed each week. Yield (Y) was calculated as $Y = [(B_t - B_0)$ (dry wt/wet wt)/tV] (DeBoer and Ryther, 1977), where B_t is the final wet weight, B_0 the initial biomass and V the algal tank volume (m^3). For dry weight-wet weight conversions, the following ratios were used (Martínez-Aragón et al., 2002): 0.161 (*U. rotundata*), and 0.137 (*G. longissima*). Data were converted to areal units of m^2.

Biofiltering calculations

The biofiltering capacity for phosphate of *U. rotundata* and *G. longissima* was calculated from the difference in phosphate concentration (μM) in the seawater outflowing the tanks as:

$$100([PO_4^{3-}]_{\text{Control}} - [PO_4^{3-}]_{\text{Algal tank}})/[PO_4^{3-}]_{\text{Control}}$$

For the calculations we assumed that biofiltration by planktonic cells and the biofilm and/or epiphytes growing on the tank's wall was similar in the algal tanks and the Control.

The estimation of the biofiltering capacity for ammonium must take into account the accumulation of total oxidised N ($\text{ToxN} = [NO_3^-] + [NO_2^-]$) due to nitrification, which must be subtracted from the difference in ammonium concentration between the control tank and the algal tank:

$$100\{[NH_4^+]_{\text{Control}} - [NH_4^+]_{\text{Algal tank}} - ([\text{ToxN}]_{\text{Algal tank}} - [\text{ToxN}]_{\text{Control}})\}/[NH_4^+]_{\text{Control}}$$

Then, the difference shown within brackets can be considered as an estimation of the ToxN production on the seaweed fronds. In this case, we assumed that nitrification in the water plus the organic film and/or epiphytes growing on the tank's wall were similar in each of the algal tank and the control tank. Finally, due to the high inflow ammonium concentration we considered that nitrate uptake by the macrophytes was negligible and therefore, *Ulva* and gracilarioids strongly preferred ammonia-N to oxidised N as in many micro and macroalgae (Dorcht, 1990; Naldi and Wheeler, 1999).

The nitrification rate on the seaweed fronds (μmol N d^{-1}), defined for practical considerations as the net accumulation rate of ToxN, was then calculated from outflow concentrations in the control and the algal tanks multiplied by the seawater flow, based on Krom et al. (1995).

Nutrient uptake rate calculations

For each sampling day, the rate of phosphate uptake (μmol PO_4^{3-} g^{-1} wet wt d^{-1}) was calculated as:

$$Q([PO_4^{3-}]_{\text{Control}} - [PO_4^{3-}]_{\text{Algal tank}})/B$$

Whereas the formula for the estimation of ammonium uptake must take into account the effect of nitrification in the seaweed tanks:

$$Q\{[NH_4^+]_{\text{Control}} - [NH_4^+]_{\text{Algal tank}} - ([\text{ToxN}]_{\text{Algal tank}} - [\text{ToxN}]_{\text{Control}})\}/B$$

Where Q represents the seawater flow (2100 L d^{-1}) and B represents the biomass each sampling day. Again, this equation was used considering the assumptions discussed above.

Chemical analysis

Triplicate water samples were collected in the morning (11 a.m.) for nutrient analysis, approximately twice a week. Samples were collected at the inflow and outflow of the tanks. In addition, water samples were collected every 2–4 h during a 34-h cycle to analyse diel variations in nutrient concentrations. Water samples were filtered through Whatman GF-F filters and stored in propilene vials at −4 °C for further nutrient analysis. Orthophosphate was determined as soluble reactive phosphorus according to Murphy and Riley

(1962). DIN analyses (ammonium, nitrate and nitrite) were carried out according to Grasshoff et al. (1983). Tissue C and N were determined using a Perkin-Elmer 240 CNH elemental analyser. Tissue P was analysed by acid digestion in triplicate samples (Sommer and Nelson, 1972).

Macroalgal chlorophyll content was extracted from fresh tissues in 4 ml of N,N-dymethyl formamide, kept in darkness overnight at 4 °C and determined according to Porra et al. (1989). Soluble proteins were determined spectrophotometrically according to Bradford (1976).

Heavy metals (Zn, Pb, As, Cd, Hg, Fe) and minerals (Ca, Mg, Na) were analysed from lyophilised dry algal samples stored in propilene vials until analysis. The lyophilised homogenised samples were acid digested by microwaves at high pressure (Milestone Ethos 1600). The digested algal samples were cooled and diluted to 50 mL. The metal concentrations in algae were determined either by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), with a model PU7000 (Phillips) or by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), with a model PU9390X (Phillips), depending on the concentration. Blanks and reference standards were run for quality assurance of the results.

Statistics

Due to the number of tanks available and other technical difficulties, including costs, in performing replication of tanks, the experiment was carried out as a simple pseudoreplication. Therefore inferential statistics was not applied between both algal tanks. Differences between means of inflow and outflow nutrient concentration within an algal tank during the system operation were tested by a two-sample student *t* test (Zar, 1984), as in this case observations were obtained from two independent experimental units. The null hypothesis was rejected at the 5% significance level.

Results

Culture conditions and algal biomass evolution

Physical and chemical variables in the control and the algal tanks were fairly stable during the experiments. Temperature varied between 18 °C in February to 20 °C in May. Mean pH and salinity during the system operation was 7.5 and 41.3 psu respectively. Dissolved oxygen was always around 100% saturation.

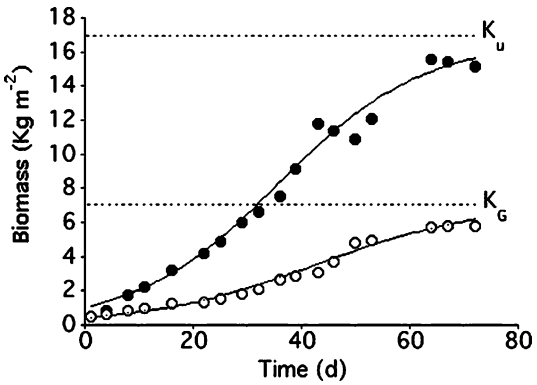

Figure 1. Biomass growth of the macroalgae *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) in a 600 L outdoor cultivation tank. Data were fitted to a logistic equation. K represents the maximum stocking density for each species.

The increase of algal biomass during the experiment is shown in Figure 1. The biomass evolution suggested suboptimal conditions at the beginning and at the end of the operation system and can be fitted to an integral form of the logistic curve $B_t = K/[1 + \exp(a–rt)]$, rearranged according to Pearl (1930), where B_t is the biomass at time *t* and *a* is a constant of integration defining the position of the curve relative to the origin. From this equation, two main parameters were calculated. The asymptotic biomass or maximum stocking density of seaweeds (K) was 16.7 kg wet weight m^{-2} (SE = 0.77) for *U. rotundata* and 7.1 kg m^{-2} (SE = 0.51) for *G. longissima*. That means approximately 27.8 g *Ulva* L^{-1} and 11.9 g *Gracilariopsis* L^{-1}. The rate of biomass growth or the intrinsic capacity for biomass increase (r) at the particular culture conditions was 0.075 d^{-1} (SE = 0.007) and 0.065 d^{-1} (SE = 0.006) for *U. rotundata* and *G longissima* respectively. Yield was also higher in *U. rotundata* (33.6 g dry wt m^{-2} d^{-1}) than in *G longissima* (10.2 g dry wt m^{-2} d^{-1}).

Phosphorus

The biofiltration efficiency for phosphate during the operation of the system is shown in Figure 2. Phosphate concentration in the inflowing water ranged from 11.3 to 24.7 μM. There was no significant variation between the inflow and the outflow water ($P > 0.2$). The biofiltration efficiency for phosphate alternated periods of negligible percentages (less than 0.5%) with others of significant biofiltration with a maximum of 26.2% in *U. rotundata* and 19.4% in *G. longissima*. The mean biofiltration efficiency of the two species

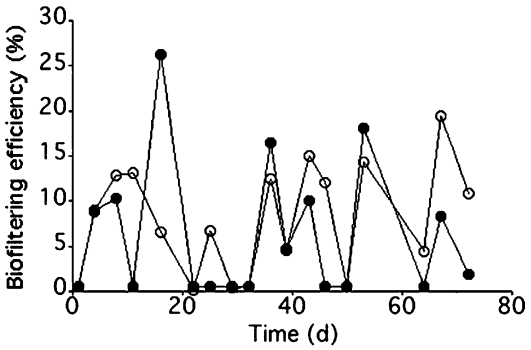

Figure 2. Phosphate biofiltering efficiency of *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) during the monitoring period.

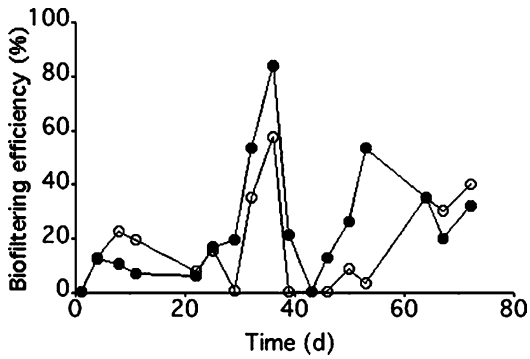

Figure 3. Ammonium biofiltering efficiency of *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) during the monitoring period.

was similar (6.08 ± 7.47% for *U. rotundata* and 7.97 ± 5.91%, for *G. longissima*). However, the mean phosphate uptake rate was higher in *G. longissima* (1.56 ± 0.403 μmol PO_4^{3-} g^{-1} wet wt d^{-1}) than in *U. rotundata* (0.637 ± 0.266 μmol PO_4^{3-} g^{-1} wet wt d^{-1}). On the other hand, the mean tissue phosphorus in the two species (3.0 ± 1.32 mg g dry wt^{-1} in *U. rotundata*; 3.49 ± 0.67 mg g dry wt^{-1} in *G. longissima*) was always higher than critical values (the tissue P concentration needed to sustain maximum growth) reported for these species, indicating that phosphate was not limiting growth. The P biomass (per dry wt) in the cultures increased from 0.120 g to more than 7 g in *U. rotundata* and from 0.216 to more than 2.5 g in *G longissima*. From these values and the estimation of the total phosphate input to the macroalgal tanks it can be deduced that overall, the *Ulva* tank removed 8.9% of the total phosphate input to the system while the estimation of this percentage in the *Gracilariopsis* tank was 3.2%. These values were similar to the mean biofiltration efficiencies measured in these species (see above).

Nitrogen

Figure 3 shows the biofiltration efficiency for ammonium. Efficiencies ranged from negligible percentages up to 85% in *U. rotundata* at the end of March, when seaweed density was half maximal. Overall, efficiencies increased smoothly as algal biomass approached the maximum stocking density of the cultivation tank. Both algae showed similar biofiltering efficiencies, with mean biofiltration of 24.4 ± 5.19% and 19.1 ± 4.07% for *U. rotundata* and *G. longissima*, respectively. The inflow ammonium concentrations varied from 24

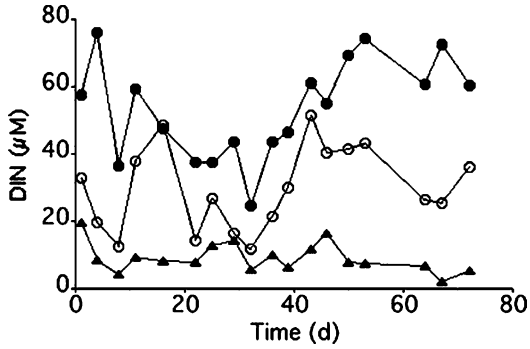

Figure 4. Inflow DIN concentration in the *Ulva* and *Gracilariopsis* cultivation tanks. Nitrite: triangles, nitrate: empty circles, ammonium: filled circles. Data are means of 3 replicates and error bars (SE < 1%) were omitted for a better clarity.

to 76 μM and usually represented the main form of DIN (Figure 4). Inflow and outflow ammonium concentrations differed significantly ($P < 0.05$), as ammonium inflowing the cultivation tank showed diel fluctuations (see below). As with phosphate, ammonium uptake rate during the system operation was higher in *G. longissima* than in *U. rotundata* (mean rates 13.8±3.15 μmol NH_4^+ g^{-1} wet wt d^{-1} and 5.99 ± 1.78 μmol NH_4^+ g^{-1} wet wt d^{-1}, respectively).

The production of ToxN in the seaweed tanks, considered to be the nitrification rate occurring on the fronds due to nitrifying bacteria that settled on them, was much lower (less than 50%) than ammonium uptake rates by the seaweeds, suggesting that, overall, algae competed efficiently for ammonia against the nitrifyers. ToxN accumulation on the seaweed surfaces increased during the monitoring period from negligible values up to 49.3 mmol N d^{-1} in *U. rotundata* and 19.3 mmol N d^{-1} in *G. longissima* at the end of

the study period. These maximum rates corresponded to less than 24% of the daily DIN input to the algal tanks. On the contrary, specific rates of nitrification were fairly constant throughout the study period. Mean specific nitrification rates were higher on the surface of *G. longissima* ($6.51 \pm 1.56\,\mu$mol N g^{-1} wet wt d^{-1}) than on *U. rotundata* ($2.65 \pm 0.524\,\mu$mol N g^{-1} wet wt d^{-1}).

The inflow nitrate concentrations ranged from 11.7 to 51 μM (Figure 4) and, as occurred with ammonium, there were significant differences between inflow and outflow nitrate in the seawater (data not shown).

Nitrite was generally the DIN form at the lowest concentration (Figure 4), being always higher in the algal tanks than in the control (data not shown). In addition, the difference in nitrite concentration between the algal tanks and the control tank increased as the algal biomass increased in the culture (data not shown), suggesting again that nitrite was accumulating in the system due to incomplete bacterial nitrification. Inflow nitrite concentration ranged between 2.1 μM to 16.3 μM, with mean values similar to the seawater outflowing ($P > 0.2$).

The nitrogen content of algal biomass in the two species increased steadily during the monitoring period (Figure 5). Furthermore, tissue N concentrations were always higher than critical N values reported for these species, indicating that nitrogen was not limiting growth. In addition, the C:N and the N:P atomic ratios in the biomass suggested no nutrient limitation (data not shown). The total N biomass (per dry wt) in the cultures increased from 2.3 g to c.a. about 115 g in *U. rotundata* and from 2.8 to nearly 38 g in *G. longissima*. From these values and the estimation of the total DIN input to the macroalgal tanks during the system operation it can be concluded that overall, the *Ulva* tank removed 54% of the total DIN input while *Gracilariopsis* removed 17%. These values are in the same order of magnitude of the mean biofiltration efficiencies measured in these species (see above).

Diel biofiltration cycles

In April, inflow and outflow nutrient concentration was also measured during a 34 h period to assess whether biofiltration by algae showed diel fluctuations. Inflow and outflow phosphate concentrations were similar and did not show any fluctuation (data not shown). On the contrary, DIN showed diel fluctuations (Hernández et al., in press). Inflow ammonium concentrations reflected the feeding period as they were lower early in the morning and higher in the afternoon, whereas outflow concentrations decreased during the afternoon. Inflow nitrate concentrations declined during the night, while outflow concentrations reflected the nutritional history of the tank and the accumulation of nitrate from ammonium due to nitrification, with a lag period of two hours approximately. Finally nitrite concentrations evidenced smooth diel fluctuations but no defined trend was observed.

From these values the biofiltration efficiency was estimated. Phosphorus was biofiltered at a low percentage, as mean values were lower than 4.5% in the two species (Figure 6). Percentages ranged from negligible values (less than 0.5%) to a maximum of 14.3% in *G. longissima*. In addition, no defined pattern was observed during the daily evolution.

On the contrary, ammonium biofiltration efficiency seemed to decrease early in the morning and by

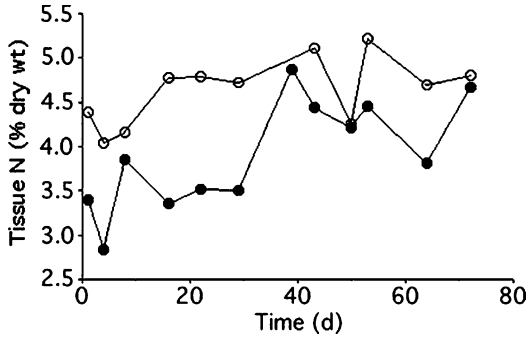

Figure 5. Tissue N in *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) during the monitoring period. Data were obtained from a pooled sample of 3 separate plants.

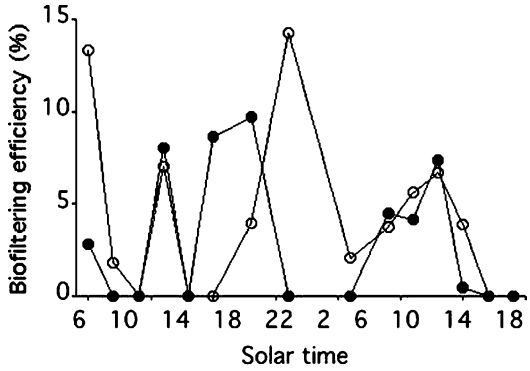

Figure 6. Phosphate biofiltering efficiency of *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) during a diel cycle.

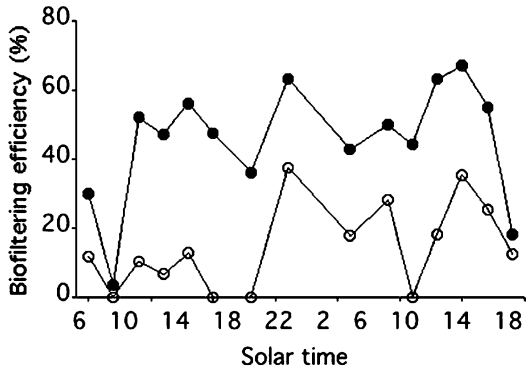

Figure 7. Ammonium biofiltering efficiency of *Ulva rotundata* (filled circles) and *Gracilariopsis longissima* (empty circles) during a diel cycle.

late afternoon (Figure 7). Ammonium was biofiltered efficiently, especially in *U. rotundata* where percentages up to 67% were observed. Biofiltration efficiency during the 34 h period was lower in *G. longissima*. Hence, mean ammonium biofiltration efficiency was higher in *U. rotundata* (45.1 ± 16.8%) than in *G. longissima* (14.5 ± 12.2%). During the 34-hours cycle, mean nitrification on the seaweed surface represented less than 8% of the DIN input to the algal tanks. In addition, mean nitrification rates occurring on the fronds represented less than 18% of the ammonium uptake rate by *U. rotundata* but a great percentage (85%) of the ammonium uptake rate by *G. longissima,* suggesting that, at least during certain periods, this species was not competing efficiently for ammonia against the nitrifyers, specially when biofiltration efficiency was lowest.

Algal composition as economic source

Concentrations of some metals in the macroalgal tissue are shown in Table 1. Samples for analysis were collected at the end of the monitoring period so that metal accumulation in the macroalgal tissue during the system operation would be expected. Except for Ca and Mg, *U. rotundata* exhibited higher metal content than *G. longissima*. Values suggested no heavy metal contamination of tissues and the ability to use the cultivated plants in the food industry. Table 2 shows the elemental nutrient C:N:P composition of *U. rotundata* and *G. longissima* at the end of the system operation. The C:N and N:P ratios indicated that macroalgal growth was not nutrient limited.

Table 1. Content of some metals and heavy metals in *Ulva rotundata* and *Gracilariopsis longissima* at the end of the monitoring period. Biomass is expressed by dry wt.

Metal	Units	*U. rotundata*	*G. longissima*
Ca	% wt/wt	0.208 ± 0.002	0.615 ± 0.006
Mg	% wt/wt	2.56 ± 0.01	3.24 ± 0.05
Na	% wt/wt	7.08 ± 0.12	0.412 ± 0.004
K	% wt/wt	14.8 ± 0.2	2.51 ± 0.04
Fe	mg Kg^{-1}	260 ± 9	109 ± 10
Zn	mg Kg^{-1}	50.0 ± 0.6	43.6 ± 0.5
Pb	mg Kg^{-1}	0.194 ± 0.012	0.178 ± 0.014
As	mg Kg^{-1}	2.08 ± 0.07	2.00 ± 0.09
Cd	mg Kg^{-1}	0.297 ± 0.012	0.198 ± 0.014
Hg	mg Kg^{-1}	u.d.l.	u.d.l.

Data are mean of three replicates ± SD. U.d.l.: under detection limits.

Table 2. Tissue elemental composition in *Ulva rotundata* and *Gracilariopsis longissima.*

Element/compound	Units	*U. rotundata*	*G. longissima*
C	% dry wt	28.7	31.4
N	% dry wt	4.67	4.80
P	% dry wt	0.358 ± 0.102	0.339 ± 0.073
C:N (atomic ratio)		7.17	6.63
N:P (atomic ratio)		28.8	31.3

Data are mean of three replicates ± SD. C and N values were obtained from pooled samples of 3 separate plants.

Discussion

Culture conditions and algal biomass evolution

The approach to the maximum stocking density of *Ulva* and *Gracilaria* under the culture conditions was probably governed by thalli self-shading, as nutrient limitation in the cultivation tanks was unlikely according to tissue nutrient concentrations. Photon irradiances at the beginning of the experiment (February) are rarely limiting at the site, as daily irradiance was about 12 MJ m^{-2} d^{-1} (Hernández et al., 1997), with photon irradiance easily reaching 1500 μmol photons m^{-2} s^{-1} at midday. As irradiance increased during the time of cultivation, it compensated in part the self-shading caused by the increase in biomass, especially as the saturation irradiance for the two species is about 150 μmol photons m^{-2} s^{-1} (Vergara et al., 1997; Andría 2001) and then light is in excess for photosynthesis (Long and Humpries, 1994). However, mean incident light decreased while biomass accumulated in the tanks. For

instance, according to a biomass per *Ulva* layer of 20 g dry wt m^{-2} (Hernández et al., 1997), the maximum stocking density under the culture conditions gave a Thallus Area Index of 134 approximately. Even if algae are continuously moving while kept suspended in the water, light limitation by self-shading is highly probable.

Growth however depended on many other factors. For instance, maximum growth rate of *U. rotundata* in the field occurs at the end of September and is fairly constant from February to June (Hernández et al., 1997). On the other hand, *G. longissima* populations are subjected to large seasonal variations. Growth rates are maximal in April, June and September but decrease in May and are negative in June, when excess light and temperature may lead to physiological damage (Pérez-Lloréns et al., 2004).

It is well known that *Ulva* and gracilarioids prefer ammonium-N to nitrate-N, especially under high ammonium concentration (Wallentinus, 1984, Krom et al., 1995; see also Naldi and Wheeler, 1999). In our system, inflow ammonium concentration was always higher than 24 μM, which assure that little or negligible nitrate was taken up by the algae even when the culture reached the maximum stocking density, which appears to be imposed by self-shading and not by nutrient limitation.

Ulva *and* Gracilariopsis *as biofilters*

Water self-purification in aquatic ecosystems is based on a number of interconnected processes either physical, chemical or biological (Ostroumov, 2002). Integrating aquaculture represents a promising solution to recycle the waste of intensive aquaculture practises (e. g. Neori and Shpigel, 1999; Naylor et al., 2000). It was found in a laboratory study that *U. rotundata* and *G. longissima* biofiltered efficiently the dissolved nutrients from a sea bass cultivation tank so that integrated systems involving these species may be scaled up (Hernández et al., 2002; Martínez-Aragón et al., 2002). The outdoor cultures at mesoscale level assayed in the present study suggest that these species have a significant buffering ability for ammonium and phosphate concentrations in the system. In addition, the algal biomass can add revenue to the fish farm due to its chemical composition. The algal tissue can be utilised in various ways for nutritional purposes and/or as an agar source. At the same time, the cultivation system provides a practical solution to major management and environmental problems of land-based mariculture.

The present study showed that the main process removing dissolved nutrients from the seawater was uptake by seaweeds. The mean N and P fluxes (1.13 g PO_4^{3-} d^{-1}; 2.94 g DIN d^{-1}) are in the range of fluxes reported in previous studies (Cohen and Neori, 1991; Krom et al., 1995). However, according to these, the seawater flow used in our study may be increased so that higher nutrient uptake rates and higher yields could be achieved but at the cost of reduced removal efficiency (Chopin et al., 2001). For instance, Jiménez del Río et al. (1996), using an *Ulva rigida* tank biofiltering system, obtained a maximum yield of 40 g dry wt m^{-2} d^{-1} at a DIN inflow rate approximately 30% greater than in our culture conditions, similar to the yield obtained for *U. rotundata* in the present study.

The potential of *Ulva* and *Gracilariopsis* as promising candidates for the development of wastewater biofiltering integrated polycultures has been demonstrated both under laboratory and field conditions. For instance, the study by Jiménez del Río et al. (1996) showed that *U. rigida* stripped with high efficiency the dissolved inorganic nitrogen (DIN) in the wastewater effluents form a *S. aurata* cultivation tank (more than 90% at 2 volumes d^{-1} water flow). The removal efficiency decreased when water flow increased up to 12 volumes d^{-1}. More recently, Neori et al. (1998) reported a reliable filtration performance (mean removing efficiency of 34% of inorganic N input but less than 25% of phosphorus) of *U. lactuca*. In contrast, *Gracilaria conferta* was deemed unsuitable for the application in a complex integrated culture of seaweed, the invertebrate marine macroalgivore *Haliotis tuberculata* (abalone) and *S. aurata*. In addition, an integrated treatment of a shrimp effluent with oyster and macroalgae found that *Gracilaria edulis* reduced more than 95% the ammonium concentration in two hours (Jones et al., 2001). Furthermore, the ammonium uptake rate at the ammonium concentration (51 μM) was similar to the value reported in our study. Consistent with the ammonium biofiltration, phosphate was reduced by 44%.

Other studies at a larger scale have also shown that ammonium and phosphate can be biofiltered in integrated fish-macroalgae cultivation systems, establishing environmental and economic benefits with the development of such practises. Buschmann et al. (1996) reported that *G. chilensis* was able to remove about 64% of the total DIN and 32% of the dissolved phosphorus in an integrated salmon-*Gracilaria* cultivation system, even though the authors pointed out that the cultivation strategy followed in their study did

not maximise the biofiltering efficiency of this species. Troell et al. (1997) estimated that, extrapolating to a large scale, *G. chilensis* co-cultivated with salmon had the potential to remove around 6.5% of DIN and 27% of dissolved phosphorus from the fish farm effluents. It is possible to extrapolate to a commercial production level how much nutrients released by fish production can be removed. This estimation, however, is complex but it is being addressed taking into account the whole aquaculture site (Pérez-Pastor, 2005). As *G. longissima* can be cultivated on ropes at the site, with growth rates up to 6% d^{-1} (Hernández et al., in press) and *Ulva* thrives naturally, preliminary data suggest that total nutrient discharges from the farm may be significantly biofiltered. However, the revenues and extra costs involved (investments and management practices) need to be assessed.

Nitrification

Total oxidized nitrogen accumulated in the system due to bacterial nitrification on the algal fronds. The conditions necessary for this process to occur; oxygen-rich seawater and high ammonium concentration, were present throughout the monitoring period. As the temperature in the cultures increased very slightly during the system operation, the marked increase in ToxN during the study period must be related to the increase of seaweed biomass, and hence surface area. The maximum nitrification rate estimated in our study was similar to the average nitrification rate (50 mmol d^{-1}) reported by Krom et al. (1995) but much lower than the 240 mmol d^{-1} reported by Dvir et al. (1999), both in a prototype integrated mariculture system involving *U. lactuca*. However, the net nitrification rate estimated in these studies included contributions by nitrifying bacteria growing on the walls of the algal tanks and suspended particles, which were not included in our estimation (see Methods). From the comparison of ammonium uptake rates and specific nitrification rates it seems clear that, overall, the nitrifyers do not outcompete the macroalgae for the use of ammonium, as these rates represented less than 50% of the ammonium uptake rates by both macroalgal species. Krom et al. (1995), and Dvir et al. (1999) reached similar conclusions in an integrated culture of *U. lactuca* and *S. aurata*. Neori et al. (1998) found that oxidised N was sporadically produced in their algal biofiltering tanks in small quantities. However, it should be taken into account that sporadically, specific nitrification rates in *G. longissima* can be similar, or even higher than the ammonium uptake rates, as shown by the daily biofiltration cycle (Hernández et al., in press).

Algal composition

The quality criteria for macroalgal powder still need specific reference standards for many countries, including Spain. The content of some nutritional components in *U. rotundata* and *G. longissima* suggests that these species could be used for human consumption, although the use of seaweeds as sea vegetables in Southern Spain is very limited. Ca, Fe and Mg content was similar to values reported for different edible macroalgae; Ca and Fe being overall 10 times higher than common vegetables (Chapman and Chapman, 1980, Indergaard and Minsaas, 1991). *Ulva rotundata* showed a high K content, higher than many other edible seaweeds (Chapman and Chapman, 1980). On the other hand, the heavy metal content in the macroalgae fulfilled the maximum tissue concentrations allowed for human consumption in France and USA (Indergaard and Minsaas, 1991). However, according to these authors Zn, As and Cd contents would be beyond the permitted limits in Japan. Values of Pb were similar to concentrations reported for natural populations (Muse et al., 1999; Sawidis et al., 2001; Vasconcelos and Leal, 2001). The Zn content in *U. rotundata* suggests a moderate degree of contamination in the inflow waters as the range of mean zinc concentration reported in *U. lactuca* from polluted sites is 42-160 mg kg^{-1} (Brown et al., 1999), and values were in the upper range of tissue concentrations reported for algal natural populations (Muse et al., 1999; Sawidis et al., 2001; Vasconcelos and Leal, 2001).

Variations in tissue N and P reflect inherent species-specific differences in the ability to sequester nutrients. The tissue nutrient levels reached in both species are a consequence of the nutrient input, algal demand and biomass dilution due to growth rates. In the present study tissue content reflected the high potential nutrient storage of *Ulva* and *Gracilariopsis*. The tissue P and N measured at the end of the monitoring period suggests that nutrients were stored in excess of the critical limits (the tissue nutrient level needed to support maximum growth) as values were well higher than P critical concentrations reported for different macroalgae (0.13–0.24% dry wt; Delgado et al., 1996; Hernández et al., 1997; Lyngby et al., 1999). In addition, the tissue N concentrations were also much higher than critical quotes reported in these genera: 2.2% in *U. lactuca* (Pedersen and Borum, 1997), 2% in *Gracilaria*

tikvahiae (Hanisak, 1983). The C:N and the N:P ratios also support our findings as both were lower than mean ratios reported for macroalgae (Duarte, 1992).

Final remarks

The integrated system of *S. aurata* and macroalgae reduced efficiently the dissolved nutrient loading, and hence reduced adverse impacts on the environment with a concomitant increase of the water quality. In addition, the system produced a significant crop of macroalgae that is of commercial value. The cultivation of *Gracilariopsis* in the site can be developed even at a larger scale, growing vegetative cuttings on suspended braided nylon ropes (Hernández et al., in press) and therefore gaining sustainability for the integrated culture system. Therefore, at this scale it will be possible to reduce significantly the total environmental cost due to the nutrients released by the fish farm, as Chopin et al. (2001) have pointed out.

Future studies must address the development of integrated systems to further reduce the outflow nutrient concentration, in particular phosphate and nitrate, and hence to increase the water quality of the effluents. New designs using different species, tanks with different surface:volume ratios and/or various biofiltering stages should be tested to reduce the dissolved nutrient concentration in the effluents. This study pointed out how important co-culture is in performing ecosystem services leading to upgrading water quality and suggested that more ecologically sound aquaculture practises may be adopted by the local farms.

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