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Studies on the biofiltration capacity of *Gracilariopsis longissima*: From microscale to macroscale

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Abstract

The potential of the red agarophyte *Gracilariopsis longissima* as biofilter for phosphate and ammonium in effluents outflowing intensive marine fish cultures was assessed at different scales. Previous studies showed that both laboratory (microscale level) and outdoor cultivation (mesoscale level) were feasible, with a maximum sustainable yield of 270 g fresh wt m^{-2} day^{-1} approximately, at a biomass higher than that predicted in a logistic model, a deviation attributable to an improvement of the culture conditions during the monitoring period. At a mesoscale level, a 34-h cycle suggested that the nitrification rate on the seaweed fronds showed diel fluctuations, with rates peaking early in the morning, when ammonium uptake rates were negligible. Mean nitrification rates were similar to ammonium uptake rates, suggesting that nitrifyers outcompete *G. longissima* for the use of ammonium; especially when mean biofiltering efficiencies were less than 15% during the 34-h period.

G. longissima thrives naturally in different earthen ponds of a fish farm in Cádiz Bay Natural Park, Southern Spain, especially in the outflowing reservoir earthen ponds, where biomass reached values up to 278 g dry wt m^{-2} during the spring. A field cultivation system for *G. longissima* (macroscale level) was designed to find the best scenario in terms of earthen pond, season or current conditions. The best cultivation method was the growth of vegetative cuttings on suspended braided nylon ropes. The highest growth rates (up to 6% day^{-1}) and biomass (up to 10 g fresh wt cm^{-1} rope) were obtained in ponds receiving outflow waters, suggesting a nutrient effect. The net P production reached 24.9 μg P cm^{-1} rope day^{-1} and was also higher on braided nylon suspended ropes placed at the outflowing reservoir earthen ponds. A similar result was found regarding net N production. However, in this case, mean production (≈170 μg N cm^{-1} rope day^{-1}) was similar in the different earthen ponds and channels. The increase in P and N biomass suggested that *G. longissima* was biofiltering efficiently nutrient wastes from the fish farm.

The results pointed out the high potential ability of *G. longissima* to biofilter waste waters from a fish farm, encouraging a large scale cultivation of this species. Future practices using this macroalgae may be implemented in local fish farms, resulting in both environmental and economic advantages.

Keywords: Nutrient uptake; Integrated aquaculture; Ecological engineering; Nitrogen; Phosphorus

1. Introduction

Intensive fish farming activities may cause several environmental impacts widely documented in the

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literature, as habitat modification or coastal eutrophication (Read and Fernandes, 2003). Waste water discharges laden with feed wastage, fish excretion and faeces increase the organic and inorganic nutrient input to the ecosystem. These impacts tend to be most severe in areas with poor water exchange, especially if aquaculture practices are concentrated (Iwama, 1991). In this context, the promotion of more sustainable aquaculture practices to improve environmental performance has been strongly recommended (NACA/FAO, 2000; Naylor et al., 2000; Troell et al., 2003). The use of macroalgae as nutrient strippers in integrated aquaculture has been demonstrated as an excellent example of ecotechnology (e.g. Neori et al., 2004), in which the production system is designed in partnership with nature (Mitsch and Jørgensen, 1989). Species of the genus *Ulva* are usually preferred in biofiltration studies due to a high biomass production and biofiltering efficiency (e.g. Neori et al., 1996). Gracilarioid species (mainly *Gracilaria* but also *Gracilariopsis*) can also contribute to an efficient removal of dissolved P and N wastes from intensive fish farms, increasing the economic output of the activity (Buschmann et al., 1996; Troell et al., 1997; Alcantara et al., 1999; Jones et al., 2001).

In recent years, different approaches are taken by government authorities to minimize the potential environmental impacts of farm effluents. Those include effluents regulation to limit the maximum allowed nutrient concentrations in the effluent discharges, specific Codes of Conduct or appropriate Best Management Practises (Tacon and Forster, 2003). Thus, the farming industry is addressing the reduction of nutrients in the effluents.

A main issue in the effective implementation and optimal scale-up of a biofiltering system is a detailed understanding of algal ecophysiology. The optimization of the overall biofiltration efficiency necessitates a compromise between apparently conflicting aims: water flow, biomass production, nutrient uptake or reduction efficiency (Chopin et al., 2001; Troell et al., 2003).

The present study briefly reviews previous investigations carried out by our research group using *Gracilariopsis longissima* as a biofilter under laboratory conditions (microscale level), where the emphasis was focused in algal physiology (Hernández et al., 2002; Martínez-Aragón et al., 2002a) and under outdoor cultivation (Hernández et al., in press) at a mesoscale level. At this scale, the contribution of nitrification to the ammonium biofiltration during a 34-h cycle was analysed. Finally, the potential of culturing *G. longissima* at a macroscale level was assessed in earthen ponds of a fish farm. These studies may encourage sustainable integrated polyculture systems to be adopted by the local farmers of the Cádiz Bay Natural Park, an enclosed shallow ecosystem of high environmental value.

2. Materials and methods

G. longissima (Stackhouse), Irvine, Stentoft and Farnham was collected from two locations in Cádiz Bay. The laboratory experiments (microscale level) were performed with individuals collected from tidal channels in Los Toruños, a salt march located in the vicinity of the laboratory. The field experiments (mesoscale and macroscale levels) were carried out with algae harvested from pools at an intensive fish farm (ACUINOVA S.L.), near the town of San Fernando. This species has been named as *Gracilaria gracilis* in previous studies (Martínez-Aragón et al., 2002a,b; Hernández et al., 2002) due to a lack of definitive identification. However, in a recent study based on molecular identification of plastid rcbL DNA, our samples were assigned as *G. longissima* (Gurgel et al., 2003).

2.1. Microscale level

As these experiments will be just briefly reviewed, details of preculture conditions, phosphate and ammonium uptake experiments and biofiltering designs are given in Martínez-Aragón et al. (2002a) and Hernández et al. (2002).

2.2. Mesoscale level

500 g of initial stocking biomass of *G. longissima* was placed in an outdoor PVC tank of 600 L under natural conditions. An additional tank without algae was used as a control. The seawater was pumped from an earthen pond, circulated through an intensive culture (ongrowing phase) of *Sparus aurata* L., and then pumped to the algal tank at a water flow of 3.5 volumes day^{-1}. Details of the culture system are given in Hernández et al. (in press). The fishes were fed from 10 a.m. to 2 a.m. at a feeding rate of 0.021 kg feed kg fish^{-1} day^{-1}. The system operated from February 2001 to May 2001, when the macroalgae reached maximum biomass due to the limits imposed by the carrying capacity of the system under the culture conditions.

The algae were weighted weekly to determine the increase in biomass and to calculate specific growth rates. The maximum sustainable yield (MSY) under the culture conditions was calculated from the biomass evolution during the experiment. The asymptotic biomass or carrying capacity (*K*) was calculated from an integral form of the logistic curve, rearranged according to Pearl (1930).

On 17 and 18 April, water samples at the inflow and outflow were collected every 2–4 h during a 34-h cycle to analyse diel variations in nutrient concentrations. The biofiltering capacity for phosphate was calculated from the difference in phosphate concentration in the seawater outflowing the control and algal tank (Hernández et al., in press). For the calculations it was assumed that biofiltration by planktonic cells and the biofilm and/or epiphytes growing on the tank's wall was similar in the algal tank and the control. The estimation of the ammonium uptake and the biofiltering capacity for ammonium must take into account the accumulation of total oxidised N (ToxN = $NO_3^- + NO_2^-$) due to nitrification, which must be subtracted from the difference in ammonium concentration between the control and the algal tank (Hernández et al., in press). 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 the algal tank and the control. Finally, due to the high inflow of ammonium we considered that nitrate uptake by the macrophytes was negligible as *Gracilaria* species strongly preferred ammonia-N to oxidised N as in many algae (Dorcht, 1990; Naldi and Wheeler, 1999).

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

2.3. Macroscale level

During 2003, independent biomass samples of *G. longissima* were collected from different earthen ponds: incoming renewal waters, outflowing reservoirs and the waste water outflow channel. A diagram of the fish farm, with the pattern of flowing waters is given in Martínez-Aragón et al. (2002b). A 30 cm × 30 cm metallic frame was randomly thrown ($n = 3$) in each sampling station. The algal biomass was transported to the laboratory and carefully rinsed to remove mud and invertebrates. Samples were oven dried at 60 °C to express biomass as g dry wt m^{-2} sediment. From March 2001 to July 2002, the growth of *G. longissima* was assessed in the field by several seaweed cultivation methods, as the biomass increase of vegetative cuttings (a) maintained in mesh cylindrical cages free floating at 10 cm depth (spring 2001, as in Hernández et al., 1997); (b) placed on a submerged flat mesh anchored to the sediment (spring 2002) and (c) seeded (5 g per 25 cm of rope) on two different types of ropes (elongated cylindrical plastic and three-braided nylon ones) suspended in channels and earthen ponds of the fish farm (November 2001 to January 2002 and again in April to July 2002). Growth rates were calculated assuming exponential growth. Uptake rates (μg cm^{-1} rope day^{-1}), considered as the net P or N production, were calculated from differences in nutrient biomass divided by the period of cultivation (47 days). Values were expressed on the basis of tissue N and P, according to the tissue nutrient content on each sampling day.

2.4. Physical and chemical analysis

Temperature, salinity, dissolved oxygen and pH records were measured with a 3185 probe equipped with a 3800 water quality logger (YSI, Grant Instruments). Phosphate was determined as soluble reactive P, according to Murphy and Riley (1962). Dissolved inorganic nitrogen (DIN) analyses 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 P content was analysed by acid digestion in dried and grounded samples by triplicate (Sommer and Nelson, 1972). Tissue C and N was determined using a Perkin-Elmer 240 CNH elemental analyser.

Table 1
Gracilariopsis longissima: summary of mean values of several variables relating to phosphorus and ammonium biofiltration during a flow-through unstarved cultures (microscale experiments) performed under 2 volumes day^{-1} seawater flow

Nutrient	Integrated rate of nutrient uptake (μmol g dry wt^{-1} day^{-1})	Biofiltration efficiency (%)	Growth rate (% day^{-1})	mg gained in culture	C/N		N/P	
					Initial	Final	Initial	Final
Phosphate	7.02 ± 0.738	93.9	9.0 ± 1.0	0.059	8.32	8.23	27.8	49.7
Ammonium	163.5 ± 16.3	61.0	–	8.95	–	–	–	–

Data are means ± S.D. ($n = 3$).

2.5. Statistics

Differences between means of inflow and outflow nutrient concentration within the outdoor PVC algal tank and between ammonium uptake rate and ToxN production were tested by a two-sample Student *t*-test (Zar, 1984). Correlations were analysed by the Pearson coefficient. In all cases, the null hypothesis was rejected at the 5% significance level.

3. Results and discussion

The development of polyculture systems represents a promising solution by integrating the cultivation of macroalgae into finfish culture (Troell et al., 2003). These authors identified necessary research areas for future improvements in polycultures, including the understanding of biochemical processes in seaweeds and the scaling up of integrated aquaculture designs to commercial implementation (Troell et al., 2003). The present results point out that *G. longissima*, a macroalgae which have a large global market, may be a relevant candidate for the development of waste water biofiltering integrated polycultures at different scales.

Few studies are applicable to land-based farms using *Gracilariopsis* as biofilter (Hurtado-Ponce, 1993; Alcantara et al., 1999). However the taxonomy of this species is complex as it may be easily confounded with species of the genus *Gracilaria*. In fact, *G. longissima* was named *G. gracilis* in our previous studies due to a lack of definitive identification (see methods). Several studies have shown that ammonium and phosphate can be biofiltered efficiently in integrated fish-*Gracilaria* cultivation systems (Buschmann et al., 1996; Troell et al., 1997; Neori et al., 1998; Jones et al., 2001). In some studies (Troell et al., 1997), *Gracilaria chilensis* co-cultivated with salmon had the potential (extrapolating to a large scale) to remove around 6.5% DIN and 27% of dissolved phosphorus effluents from the fish farm. Nutrient removal efficiency was usually lower than integrated fish–*Ulva* systems and depended on many factors relating to the operation system, either physical, chemical or biological (Ostroumov, 2002; see below).

Table 2
Gracilariopsis longissima: mean values of several variables relating to biofiltration during the operation system at a mesoscale level

Phosphate biofiltration efficiency	3.2%
Tissue P content	0.35±0.067% dry wt
Ammonium biofiltering efficiency	19.1±4.07%
DIN biofiltration efficiency	17%
Tissue N content	4.80±0.46% dry wt
C/N ratio	6.78±0.70
N/P ratio	31.97±6.25
ToxN production (nitrification rate)	13.58±12.78 mmol N day^{-1}
Specific nitrification rate	6.51±1.56 μmol N g^{-1} wet wt day^{-1}

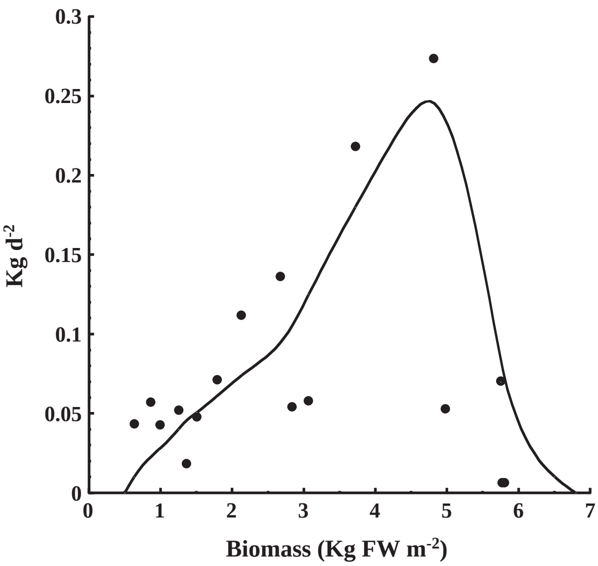

Fig. 1. *Gracilariopsis longissima*. Logistic-type model relating the biomass evolution of the culture at a mesoscale level and net biomass production to estimate the maximum sustainable yield. The logistic model would predict a MSY at 0.5 K (3.55 kg m^{-2}), but these data suggests MSY around 0.7 K (5 kg m^{-2}). Experimental points were fitted by eye.

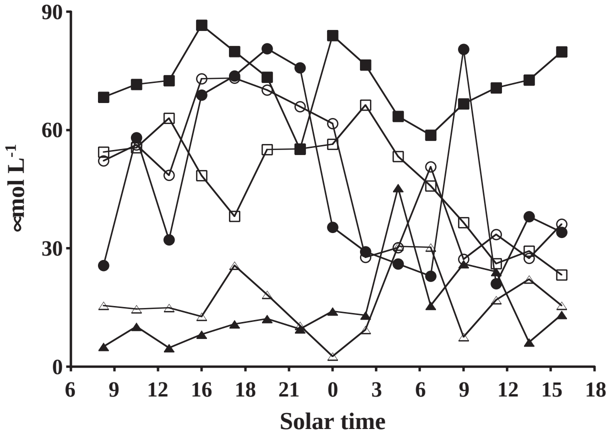

Fig. 2. Inflow (filled symbols) and outflow (empty symbols) DIN concentration in the *Gracilariopsis longissima* cultivation tank during a 34-h period. Nitrite: triangles; nitrate: circles; ammonium: squares. Data are means of 3 replicates and error bars (S.E. < 1%) were omitted for better clarity.

3.1. Microscale level

Table 1 summarizes the main results obtained in the *G. longissima* biofiltering experiments at a laboratory scale. This species removed quite efficiently the phosphate and ammonium dissolved in waste waters from a sea bass cultivation tank. After 7 h of incubation, removal efficiency of phosphate and ammonium was 62.2% and 93.2% respectively. The kinetic parameters showed that maximum ammonium uptake (21.3 ± 1.98 μmol g^{-1} dry wt^{-1} h^{-1}) was significantly higher than maximum phosphate uptake (1.25 ± 0.39 μmol g^{-1} dry wt^{-1} h^{-1}). These values were within the range of the large data set of values published for Rhodophyta (Hein et al., 1995; Lobban and Harrison, 1997; Pedersen and Borum, 1997, Jones et al., 2001).

The integrated rate of nutrient uptake was highest under 2 volumes day^{-1}, the greater seawater flow assayed in Martínez-Aragón et al. (2002a,b). Under this flow-through design, with nutrient fluxes of 6.61 ± 3.39 μmol PO_4^{3-} g^{-1} dry wt day^{-1} and 345 ± 97.6 μmol NH_4^+ g^{-1} dry wt day^{-1}, both nutrients were biofiltered at a high percentage. That suggested that the flow rate could be significantly increased. For instance, Neori et al. (1991) designed flow rates up to 16 volumes day^{-1}, rendering higher growth rates than those obtained in our study. Despite the growth rate, the tissue P content decreased during the experiment. However, due to biomass production, the net N and P biomass increased in the cultures. This production was similar to the total phosphate or ammonium input to the system. The fishpond effluent did not vary the biomass C/N ratio. However,

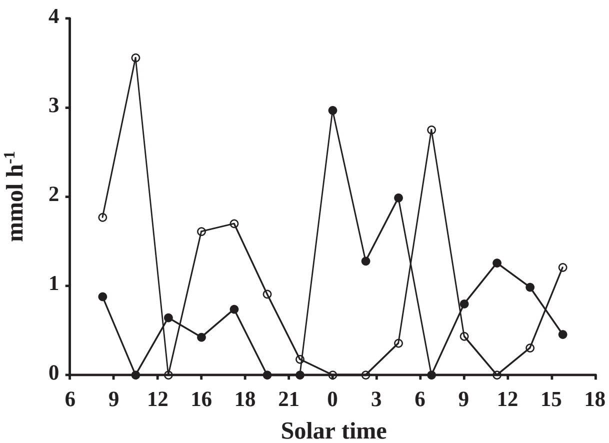

Fig. 3. Total oxidised nitrogen (empty circles) and ammonium uptake rates (filled circles) in the *Gracilariopsis longissima* cultivation tank during a 34-h period.

the N/P ratio increased at the end of the experiment. The tissue P and the increase of the N/P ratios measured at the end of the experiment rendered values below critical concentrations (the tissue P needed to support maximum growth), according to values reported for macroalgae (Duarte, 1992; Lyngby et al., 1999), suggesting that P, rather than N limited macroalgal growth. According to Chopin et al. (2001), factors as water flow and tissue elemental composition may control differences in biofiltering efficiency as it has been found in our study. Variations in elemental nutrient composition reflected inherent species-specific differences in the ability to sequester nutrients. Nutrient levels reached under the flow-through design were dependent on the nutrient flux, algal demand and biomass dilution due to growth.

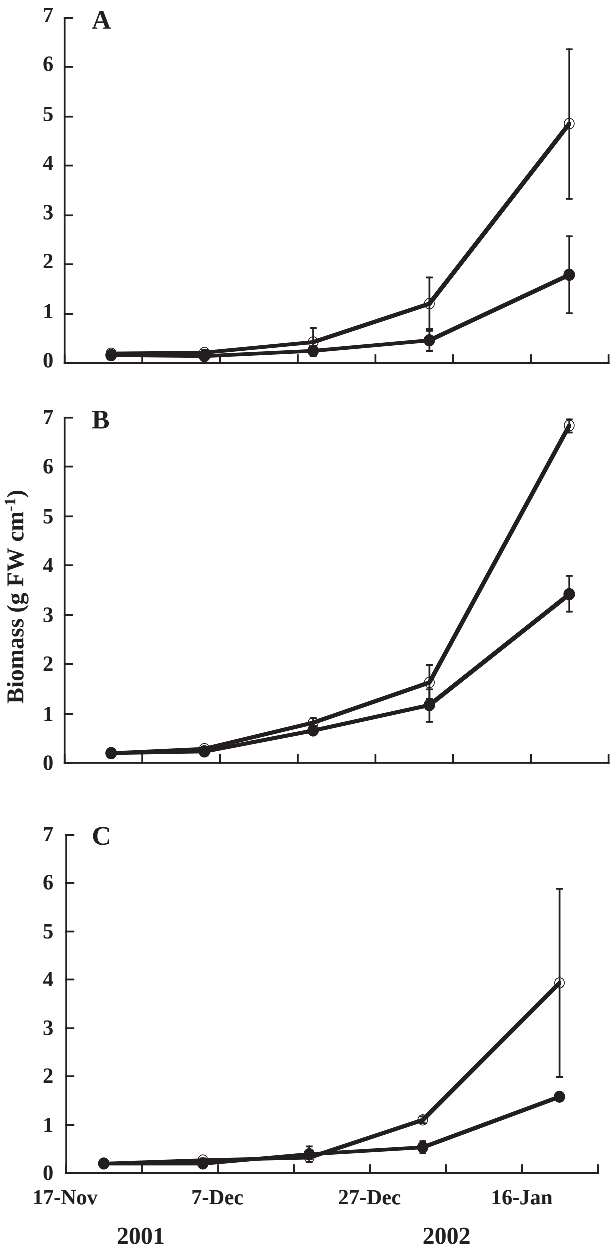

Fig. 4. *Gracilariopsis longissima*. Biomass evolution of vegetative cuttings seeded on elongated cylindrical plastic ropes (filled circles) and braided nylon ropes (empty circles) placed in different earthen ponds and channels. A) incoming renewal waters; B) outflowing reservoir; C) waste water outflow channel. Data are means±S.D. (n=2).

3.2. Mesoscale level

The biomass increase of *G. longissima* during the experiment rendered an asymptotic biomass or carrying capacity of 7.1 kg fresh wt m^{-2} (S.E. = 0.51) (Hernández et al., in press). The net biomass productivity along the experiment described an n-shaped curve (Fig. 1). From this figure it can be deduced that, under the culture conditions, the MSY (270 g fresh wt m^{-2} day^{-1} approximately) seems to occur at a biomass about 5 kg m^{-2}, a density higher than that predicted in a logistic model, which equals to 0.5 K (Krebs, 1994). This deviation may be attributable to changes in the culture conditions during the experiment, specially the increase of temperature and natural photoperiod, as water flow and pH remained constant throughout the experiment.

Table 2 shows a summary of mean values of several variables relating to the algal biofiltration during the operation system (Hernández et al., in press). *G. longissima* biofiltered phosphate and DIN at lower percentages than those observed at the microscale level which points out that nutrient uptake by *G. longissima* depends on many factors as seawater flow, nutrient loads, areal tank/biomass ratio, area/volume ratio of the tanks and many other culture conditions (Chopin et al., 2001; Troell et al., 2003). Therefore both scales are difficult to compare. This also suggests how uncertain it may be to scale up results obtained under laboratory conditions. The outdoor cultivation of *G. longissima* revealed that mean ToxN production, considered as the microbial oxidation rate of ammonia (nitirification) occurring on the algal fronds by nitrifying bacteria, accounted for nearly 44% of the ammonium uptake by the macroalgae, although in some sampling days the former may exceed the latter (Hernández et al., in press). Both N and P were always higher than critical values, indicating no nutrient limitation of growth. The same was also suggested by the C/N and N/P ratios.

As biofiltration efficiencies evidenced daily changes but without a defined diel pattern (Hernández et al., in press), it was assessed whether ToxN production showed daily changes, and therefore diminished the performance of the integrated system. Fig. 2 shows the DIN inflow and outflow concentrations during a 34-h period. There were significant differences between inflow and outflow DIN concentrations. Inflow ammonium concentration usually represented the main form of DIN and reflected the feeding period. It was lower early in the morning and higher in the afternoon, whereas outflow ammonium concentration 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 2 h approximately. Nitrite was generally the DIN form at the lowest concentration, being always higher in the algal tank than in the control (data not shown). It evidenced smooth diel fluctuations without a defined trend.

The production of ToxN in the algal tank ranged from negligible values up to 3.56 mmol N h^{-1} (Fig. 3) and overall represented less than 8% of the DIN input to the algal tank. Nitrification rates showed diel fluctuations as values peaked early in the morning and decreased during the evening. The average nitrification rate (23.7 mmol N h^{-1}) halved the average rate reported by Krom et al. (1995) in a prototype integrated system involving *Ulva lactuca*. Maximum nitrification rates occurred when ammonium uptake rates were low (Fig. 3). These rates were maxima during the night, especially at the end of the period of feeding, when inflow ammonium concentration increased (Fig. 2). The minimum ammonium uptake rates were observed early in the morning and in the evening.

There were no significant differences between mean ToxN production and mean ammonium uptake rates during the 34-h cycle, suggesting that nitrifyers may sporadically outcompete *G. longissima* for the use of ammonium, specially when mean biofiltration efficiency during the whole system operation was less than 19% in this species and slightly lower (14.5 ± 12.2%) during the 34-h period (Hernández et al., in press). Steps should be taken to diminish the microbial oxidation of ammonia, and thus increase the performance of the integrated system because a shift in NO_3^- instead of NH_4^+ does not decrease the overall DIN concentration in the effluents. These steps include the cleanliness of surfaces and appropriate ammonium loading rate (Neori et al., 2004).

Table 3
Gracilariopsis longissima: mean growth rates (% day^{-1}) obtained on the two different types of ropes and at different earthen ponds and channels

	Elongated plastic rope	Braided nylon rope	*K*
Incoming renewal waters	4.2	5.6	6.39 ± 1.49
Outflow reservoir	5.0	6.0	15.6 ± 4.10
Outflow channel	3.5	5.0	–

Rates were calculated assuming exponential growth (R^2 > 0.94). The carrying capacity for the braided nylon rope (*K*; g fresh wt cm^{-1}) was estimated in a subsequent rope seeding.

Table 4
Gracilariopsis longissima: initial and final net P biomass (μg P cm^{-1} rope) during the growth assessment on the two different types of ropes and at different earthen ponds and channels

	Elongated plastic rope			Brided nylon rope		
	Initial P biomass	Final P biomass	P biofiltration rate	Initial P biomass	Final P biomass	P biofiltration rate
Incoming renewal waters	78.7±17.4	214±89	3.08±2.07	87.7±5.4	446.6±329.1	7.63±6.88
Outflow reservoir	84.3±26.6	619±309	11.4±6.42	76.6±25.11	247±896	24.9±18.6
Outflow channel	79.0±40.9	530±146	9.61±3.98	65.4±46.1	701±83.0	13.5±0.78

Phosphorus biofiltration rate (μg cm^{-1} day^{-1}), considered as the net P production, was calculated from difference in P biomass divided by the period of cultivation. Data are means±S.D. (n=3).

3.3. Macroscale level

Biomass of *G. longissima* varied during the study period depending on the season and the earthen pond. Biomass was negligible in the waste water outflow channel throughout the study period. Within the fish farm, the lowest biomass was usually found in the incoming renewal waters. Maximum values reached 19.1 g dry wt m^{-2} during the autumn although *G. longissima* was observed only in a few sampling stations. On the contrary, *G. longissima* was found in many of the outflowing reservoirs throughout the year. Maximum biomass was usually found in spring, with biomass up to 278 g dry wt m^{-2} in some of the earthen ponds. At this time of the year, growth rates up to 8% day^{-1} have been measured in a nearby sampling station (Pérez-Lloréns et al., 2004). The lowest biomass was generally found in the autumn, when mean values reached 3.7 g dry wt m^{-2}.

Potential farm sites were tested using different cultivation methods and positioning of algae in different environments. Growth rate estimated in mesh cylindrical cages did not render satisfactory results. The rates depended on the sampling station and ranged between −0.015% and 9% day^{-1}. After 9 days of cultivation in these cages, thalli began to deteriorate. The vegetative cuttings anchored to the sediments showed low growth rates (mean values of 2.4±0.5% day^{-1}) due to light attenuation in the earthen ponds. The best cultivation method for *G. longissima* was the growth on suspended ropes (Fig. 4). Although biomass production depended on the earthen pond in which ropes were placed, the production on the nylon braided rope doubled that obtained on the plastic rope, both in the incoming renewal waters and the outflowing reservoirs and channel. Growth rates were higher in the outflowing reservoirs (Table 3), suggesting a nutrient effect as ammonium and phosphate concentration were always greater here than in the inflowing renewal waters. Another factor affecting growth was competition with other seaweeds; *Ulva rotundata* and *Enteromorpha intestinalis* hooked on the rope, especially in ponds with moderate currents. In these sampling stations, biomass production was negligible (data not shown). This inhibitory effect may include not only shading but also allelopathic effects (Friedlander et al., 2001) and should be avoided if macroscale cultivation of *G. longissima* on a commercial scale is intended.

Two months after this experiment, the nylon ropes were seeded again at the inflowing and outflowing earthen ponds and this time growth of the algae was self-inhibited when in high densities, reaching the carrying capacity of the rope. Values are also shown in Table 3. The highest carrying capacity was obtained in the outflowing reservoir (g fresh wt cm^{-1}), where the greatest growth rate was also measured again (data not shown). Overall, the possibility for a large-scale cultivation of *G. longissima* was very encouraging. However, as it has been suggested in other studies (Daves, 1995), rope systems must be carefully designed to find

Table 5
Gracilariopsis longissima: initial and final net N biomass (μg N cm^{-1} rope) during the growth assessment on the two different types of ropes and at different earthen ponds and channels

	Elongated plastic rope			Brided nylon rope		
	Initial N biomass	Final N biomass	N biofiltration rate	Initial N biomass	Final N biomass	N biofiltration rate
Incoming renewal waters	861±203	3646±320	59.2±21.1	1107±34	9412±276	176±8.02
Outflow reservoir	886±355	5111±2003	66.6±31.2	658±46.5	8509±3729	166±77.3
Outflow channel	912±466	7513±2068	140±53.9	794±549	10536±968	167±78.4

Nitrogen biofiltration rate (μg cm^{-1} day^{-1}), considered as the net N production was calculated from difference in N biomass divided by the period of cultivation. Data are means±S.D. (n=3).

Table 6
Gracilariopsis longissima: mean tissue N/P ratios at the initial and end of the growth assessment on the two different types of ropes and at different earthen ponds and channels

	Elongated plastic rope		Braided nylon rope	
	Initial	Final	Initial	Final
Incoming renewal waters	24.1±0.364	26.4±0.232	28.0±0.882	24.7±2.14
Outflow reservoir	25.4±1.88	27.2±10.2	26.3±4.24	28.8±11.2
Outflow channel	25.6±0.220	31.3±0.001	27.4±0.729	33.3±0.892

Data are means±S.D. (n=3).

the best scenario in terms of earthen pond, season and/or current conditions.

Previous studies of cultivation of *G. gracilis* suspended on ropes suggested that nitrogen wastes from a fish farm promote a faster macroalgal growth than in a control site, and that there is considerable uptake of fish-waste nutrients (Anderson et al., 1999). The initial P content of *G. longissima* ranged between 0.336% and 0.460% dry wt (Table 4), whereas the initial N content ranged between 3.90% and 5.05% dry wt (Table 5). The tissue nutrient content usually decreased slightly during the algal cultivation in both types of rope (data not shown). Values, however, were always greater than critical quotas (Hanisak, 1983), which suggest a nutrient dilution in the new biomass. Moreover, as the biomass increased on the suspended ropes, the net P and N biomass increased in the ropes (Tables 4 and 5), indicating that both phosphate and ammonium were being uptaken by *G. longissima*. Net nutrient production was always higher in the braided nylon rope. The data also suggested that maximum biofiltration rate occurred in the outflowing reservoir, where nutrient concentration was higher.

The N/P ratio (Table 6) pointed out that during the 47 days of cultivation, algae in the outflow reservoirs and channel were preferentially enriched in nitrogen. N/P ratio at the beginning of the experiment indicated no nutrient limitation (Hernández et al., 2002). Despite the increase of the N/P ratio during the cultivation, nutrient limitation was unlikely, as shown by the tissue nutrient contents and N/P ratios up to 49 for *G. longissima* under marked phosphorus limitation (Hernández et al., 2002).

Still, we are estimating the overall biofiltration by macroalgae in the fish farm, taking into account the biomass contribution by other species (mainly *U. rotundata*), nutrient input to the system and the water flow. Preliminary data suggests that macroalgae are contributing significantly to the nutrient biofiltration in the fish farm.

4. Conclusion

The polyculture of fish and *G. longissima* at different scales may deplete efficiently dissolved nutrient loads and hence adverse impacts on the environment, leading to upgrading water quality of the effluents. In addition, the system produced a significant crop of macroalgae that is of commercial value. Studies at mesoscale level pointed out that nitrification may substantially affect ammonium biofiltration, at least during certain periods. The cultivation of *G. longissima* by vegetative propagation is feasible at a macroscale level, so that high crop of macroalgae that is of commercial value can be obtained. The study suggested that more ecologically sound aquaculture practices may be adopted by the local farms. It is feasible to design promising, integrated system approaches for the management of aquaculture in southern Spain.

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