SCI. MAR., 58(4): 329-335 SCIENTIA MARINA 1994

Optimization of yield and biofiltering efficiencies of *Ulva rigida* C. Ag. cultivated with *Sparus aurata* L. waste waters*

MIGUEL JIMENEZ DEL RIO[1], Z. RAMAZANOV[2] and G. GARCIA-REINA[1].

[1] Institute of Applied Algology, Universidad de Las Palmas G.C., Box 550 Las Palmas, Islas Canarias, Spain.
[2] Department of Botany, Louisiana State University, Baton Rouge, LA, USA, 70803.

SUMMARY: *Ulva rigida* C. Ag. was cultivated in 750 l tanks at different densities and flow rates with waste waters from Sparus aurata cultivation systems, to estimate the conditions for optimizing biofiltering efficiency and yield. To maximize both characteristics: i) seaweed density should be adjusted to 2.7 g FW l^{-1} (1.2 kg m^{-2}) and ii) NH_4^+-nitrogen flow should be (at least) 150 mmol m^{-2} d^{-1}. Such conditions will yield 27 g DW m^{-2} d^{-1} with uptake efficiencies of 76 % of the daily input in our system at 12 volumes d^{-1} (110 mmol m^{-2} d^{-1}) at seaweed : fish biomass ratio of 1:4. NH_4^+ removal efficiencies would probably be increased if fish were feed as early as possible in the morning to provide maximum NH_4^+ concentrations during the light period, as uptake efficiencies are higher than in the dark.

Keywords: *Ulva rigida*, *Sparus aurata*, biofiltering, ammonia uptake, aquaculture

Abbreviations: ANE= Apparent Nitrogen Efficiency; RNE= Real Nitrogen Efficiency; PFD= *p*hoton *f*lux *d*ensity; DW= *d*ry *w*eight; FW= *f*resh *w*eight

RESUMEN: OPTIMIZACIÓN DEL RENDIMIENTO Y DE LA EFICIENCIA DE BIOFILTRACIÓN DE *ULVA RIGIDA* C. AG. CULTIVADA EN AGUAS EFLUENTES DE CULTIVO DE *SPARUS AURATA* L.– *Ulva rigida* C. Ag. fue cultivada en tanques de 750 l a diferentes densidades y tasas de renovación con efluentes de sistemas de cultivo del pez *Sparus aurata* L. con la finalidad de determinar las condiciones que permitieran optimizar tanto las eficiencias de biofiltración como la producción de algas. Para optimizar ambas características: i) la densidad de algas debería ajustarse a 2.7 g FW l^{-1} (1.2 kg m^{-2}) y ii) los flujos de amonio deberían ser de al menos 150 mmol m^{-2} d^{-1}. Bajo tales condiciones se podrían obtener producciones de 27 g PS m^{-2} d^{-1} con eficiencias de incorporación de amonio del 76% del amonio que entra al sistema a tasas de renovación de 12 vol d^{-1} (110 mmol m^{-2} d^{-1}) a una relación algas:peces de 1:4. La eficacia de incorporación de amonio podría incrementarse adelantando tanto como fuese posible el horario de comida de los peces para obtener las máximas concentraciones de amonio durante el día, coincidiendo con el periodo de máxima capacidad de incorporación del mismo por las algas.

Palabras clave: *Ulva rigida*, *Sparus aurata*, biofiltración, incorporación de amonio, acuicultura.

INTRODUCTION

Waste waters resulting from intensive fish cultivation systems contain large amounts of dissolved metabolites, mostly ammonia, generated from excretory products and bacterial ammonification of organic matter (KROM *et al.*, 1985a; PORTER *et al.*, 1987; KROM and VAN RIJN, 1989). Ammonia can increase to toxic levels, up to 450 µM, in fishponds with low or intermediate water exchange rates (PORTER *et al.*, 1987; KROM *et al.*, 1989), producing

*Received September 1, 1993. Accepted June 2, 1994.

hypereutrophic conditions due to the fertilizing effects on marine phytoplankton (KROM *et al.*, 1985a; 1985b; NEORI *et al.*, 1989). From the point of view of minimum environmental impact, recycling or recirculation systems of aquaculture would be ideal, as there would be only reduced discharges into open water bodies. The development of such systems requires the removal of solid compounds and dissolved metabolites contained in outflowing water. Solids can be easily removed by filtration or other mechanical processes but removal of dissolved metabolites requires more complex and expensive processes.

The use of seaweeds as biofilters to remove dissolved nitrogen from fish pond effluent has been reported previously (DE BOER and RYTHER, 1977; FRALICK, 1979). Species of the genus *Gracilaria* and *Ulva* have been tested, showing high growth rates and high ammonia removal efficiencies (HARLIN *et al.*, 1979).

Ulva species not only show a higher N-removal capacity than *Gracilaria* but also a higher resistance to epiphytes. Ammonia removal efficiencies of up to 85%, independently of light or temperature fluctuations, were obtained with *Ulva lactuca* L. cultivated with effluents of the marine fish *Sparus aurata* at water exchange rates of 8 volumes per day and NH_4^+ concentrations of 10-20 µmol l^{-1} h^{-1} (VANDERMEULEN and GORDIN, 1990). Sustained *Ulva* yields over 30 g DW m^{-2} d^{-1} have been reported by several authors (VANDERMEULEN and GORDIN, 1990; NEORI *et al.*, 1991).

Seaweed stocking densities, water exchange (NH_4^+-nitrogen flow through tanks) and fish:seaweed biomass rates are key factors affecting growth rate, yield and biofiltering efficiency. Those variables are usually not clearly stated in the literature. Different optimum densities for maximum yield in tank cultures have been given for different species (*Ulva* = 0.8 kg FW m^{-2}, LAPOINTE and TENORE, 1981; *Gracilaria*= 2-3 kg FW m^{-2}, LAPOINTE and RYTHER, 1978). However, higher stocking densities than those given for maximal yield in *Ulva* have been recently reported for maximal ammonium uptake and tissue nitrogen content (NEORI *et al.*, 1991).

The present study was conducted to determine the interaction between NH_4^+ flow, seaweed density and seaweed:fish biomass ratio in a co-culture system, to maximize biofiltering efficiencies and seaweed yield.

MATERIAL AND METHODS

Plant material and culture conditions

Plants of *Ulva rigida* collected from the east coast of the island of Gran Canaria (Canary Islands, Spain) were precultivated for one month under continuous wastewater flow. Necrotic or epiphytized plants were removed during this period. Preculture and experiments were conducted under greenhouse conditions in 750 l (1.75 m^2) semicircular tanks 80 cm deep. Algae were grown suspended in the water column with the aid of air diffusers located at the bottom of the tank.

Fishpond effluent was pumped from a 1 m^3 reservoir tank (washout of sediments every two weeks) connected to the output of six 1 m^3 tanks with approximately 12 kg of *Sparus aurata* in each. The water exchange rate was 8-10 vol d^{-1} per seaweed tank. Water passed through seaweed tanks was directly released to the sea.

Noon average PFD during experiments was 710 ± 80 µmol m^{-2} s^{-1}, with a daily water temperature fluctuation of 25.6 ºC (at noon) to 19.9 ºC (during the night). Differences in temperature between tanks did not exceed 0.5 ºC in each experimental set.

Experimental design

Three sets of experiments (1, 2 and 3, Table 1) each in triplicate were conducted during a two week period to estimate the effects of: i) stocking densities (1, 2 and 4 g FW^{-1} l^{-1}) and ii) wastewater exchange rates (4, 8 and 12 vol d^{-1}) on: i) NH_4^+-removal efficiency (N-efficiency) and ii) seaweed yield. Two control experiments were carried out simultaneously to estimate i) yield in tanks under continuous flow of seawater with weekly pulse-feeding (expt. 4) and ii) NH_4^+ losses of aerated culture tanks (expt. 5). In experiment 4, water supply was turned off once a week and tanks were supplied with ammonium chloride and sodium orthophosphate to a final concentration of 2.0 mM NH_4^+ and 0.15 mM PO_4^{3-} (following the concentrations used in tank cultures of *Ulva lactuca* by DEBUSK *et al.*, 1986). After 24 h, the water supply was turned on and adjusted to a flow rate of 8 vol d^{-1}

Inflow and outflow of NH_4^+ were measured as follows: i) every three hours (24 h period) the day after algae were re-stocked to their initial density and, ii) at 21:00 daily. PFD, temperature and pH values were taken simultaneously

TABLE 1.- Effect of stocking density and NH_4^+flow on ammonia removal efficiencies, growth rate and yield of *Ulva rigida*.

Exp. nº	Exch. rate (d^{-1})	Density g FW l^{-1}	NH_4^+ inflow (mmol m^{-2} d^{-1}) ± SE total	L*	D*	NH_4^+outflow (mmol m^{-2} d^{-1}) ±SE	NUR ±SE	NUE (%) total	L*¹	D*¹	Growth rate (%d^{-1})	Yield g DW m^{-2} d^{-1} ±SE
1	4	1	48.2±3.4	19.3±1.4	28.9±2.0	7.2±0.3	40.9±2.8	85.0	92.9	76.0	8.7	10.5±0.7
	4	2	48.2±3.4	19.3±1.4	28.9±2.0	4.2±0.3	43.4±3.2	90.4	97.2	85.9	6.2	13.8±0.4
	4	4	51.4±2.0	20.6±0.8	30.8±1.3	3.4±0.3	47.9±1.9	93.3	98.6	90.2	3.0	11.9±1.0
2	8	1	98.3±4.1	40.0±1.7	58.3±2.5	29.8±2.4	68.7±2.4	69.6	78.4	58.5	13.2	19.2±1.7
	8	2	107.4±3.0	43.0±1.2	64.6±1.9	20.9±1.4	86.5±2.2	80.5	85.0	63.7	10.4	27.0±0.4
	8	4	107.4±3.0	43.0±1.2	64.6±1.9	13.4±0.7	93.9±2.5	87.4	96.5	82.3	5.0	21.2±0.8
3	12	1	154.6±3.9	68.5±1.6	86.1±2.3	70.5±1.5	85.0±2.1	55.0	63.2	40.0	14.0	21.0±0.6
	12	2	154.6±3.9	68.5±1.6	86.1±2.3	44.7±1.5	110.2±2.8	71.3	79.7	55.6	11.0	29.2±0.8
	12	4	157.7±2.6	69.8±0.9	88.8±1.5	37.5±1.0	120.7±2.3	76.6	92.3	67.9	5.6	24.1±0.5
4	8	1									12.6	17.9±0.4
	8	2									9.6	24.3±0.5
	8	4									5.4	23.6±0.5
5	8	0	107.4±3.0			106.1±2.2						

L*= light period; D*= dark period; ¹= % of the total NH_4^+ (100%) which enters the system during the respective periods.

Harvesting and growth rate determination

Ulva rigida plants were harvested weekly, spinned at 2,800 rpm in a domestic clothes centrifuge (Miele WZ268) for 30 s and re-stocked to the initial density. Specific growth rates (μ) were calculated according to the equation ($\mu = 100 \ \mathrm{Ln}(W_t/W_0)/t$) by D'ELIA and DEBOER (1978), where W_0= initial fresh weight; W_t= final fresh weight; and t= time (days). A sample of 10 g was removed and dried at 80 ºC to constant weight to obtain the wet weight/dry weight ratios. Yields were calculated according to the equation ($Y = [(N_t-N_0)/t * (DW/FW)] / A$) by DEBOER and RYTHER (1977), where N_t= initial fresh weight, N_0= final fresh weight and A= area in m^2.

Analytical measurements

Ammonia analysis was carried out using a Flow Injector Analyzer (FIAstar 5010 Analyzer, Tecator, Sweden), through the gas diffusion method (RONNESTAD and KNUTSEN, 1991). PFD was measured with a LI-COR LI-1000 Datalogger using a spherical quantum sensor to estimate the decrese of PFD into the tanks, at half depth (40 cm) and at the bottom (80 cm). A plane quantum sensor was used to measure the irradiation just above the surface of the tanks. The pH and temperature values were measured with a HI 9025 microcomputer pH meter (Hanna Instruments).

Nitrogen uptake efficiencies (NUE) shows the average reduction (%) in ammonia concentration. Nitrogen uptake rate (NUR) shows the amount of ammonium removed per unit of time. The light period for the calculation of NUE, NUR and NUR per unit of biomass was from sunrise (7:30) to sunset (20:30).

RESULTS

Stocking density

According to the results of several authors for *Ulva* spp. (LAPOINTE and TENORE, 1981), maximum *Ulva rigida* yields were obtained at an initial stocking density of 2 g FW^{-1} l^{-1} (0.86 kg FW m^{-2}), with declining yields at both lower and higher stocking densities at any water exchange rate (Table 1). Densities of 1, 2 and 4 g FW l^{-1} reduced PFD by 58.3, 75.6 and 95.4% at half depth of the tank (40 cm) and by 62.2, 93.3 and 100% at the bottom of the tank (80 cm), respectively.

As observed from Fig. 1 B, increments in stocking densities can also be related to reductions in inorganic carbon concentrations or high pH, caused by photosynthetic carbon assimilation, specially in low water exchange rate culture tanks.

Growth rate and Yield

Both the growth rate and yield at 12 vol d^{-1} were twice those observed at 4 vol d^{-1} at all densities

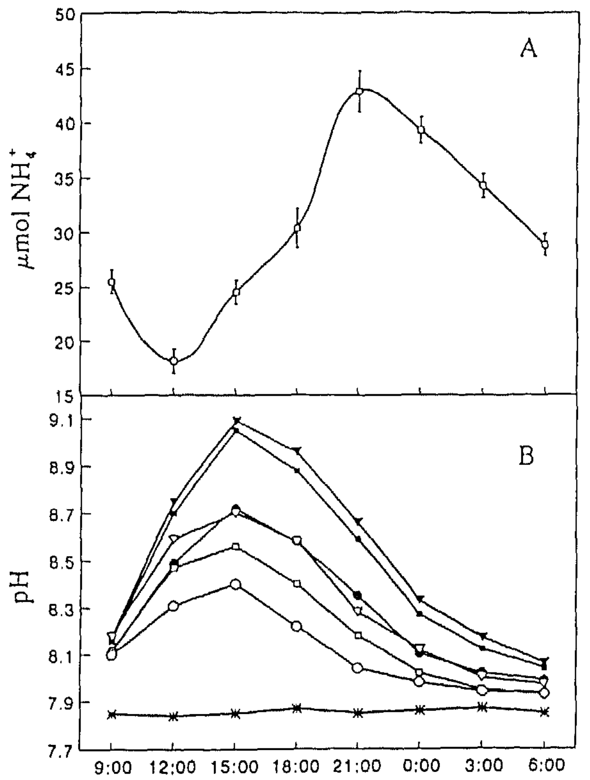

FIG. 1.-A: Daily ammonia concentration in the water of the fishpond effluent flowing into the tanks. Bars represent standard deviations (n=3). B: Daily pH evolution in: fish pond effluents (*) seaweed culture tanks at densities of 1 g l^{-1} (●), 2 g l^{-1} (■) and 4 g l^{-1} (▲) at 4 (closed symbols) and 12 (open symbols) daily water exchange rates.

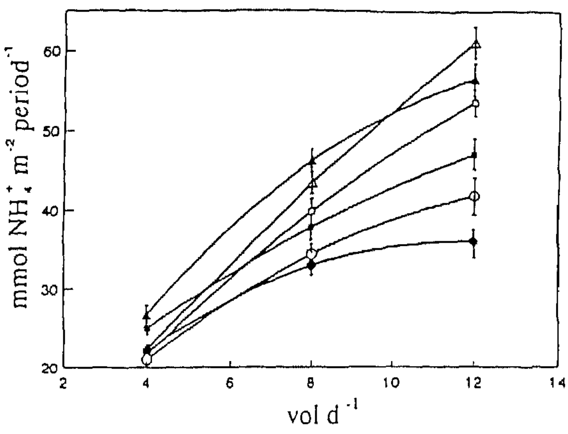

FIG. 2.- NUR during light (open symbols) and dark (closed symbols) periods at 4, 8 and 12 vol d^{-1} and densities of 1 g l^{-1} (●), 2 g l^{-1} (■)nd 4 g l^{-1} (▲). Bars represent standard deviation (n=3).

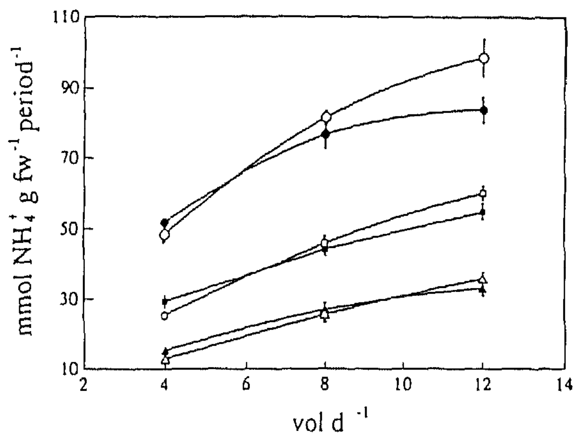

FIG. 3.- NUR per unit of biomass during light (open symbols) and dark (closed synbols) periods at 4, 8 and 12 vol d^{-1} and densities of 1 g l^{-1} (●), 2 g l^{-1} (■) and 4 g l^{-1} (▲). Bars represent standard deviation (n=3).

(Table 1). However, growth rate and yield at 8 and 12 vol d^{-1} were quite similar. Similar growth rates and yields were obtained between weekly-pulsed cultures with continuous flow of wastewater (Table 1). Disintegration of the plants and decrease in weight were observed in running through seawater tanks (non-fertilized cultures, data not shown).

Effect of water exchange rates

One of the main effects of the increase in water exchange rates were the increment in the amount of NH_4^+ and inorganic carbon provided to the plants per unit of time. Flow rates of 4 and 8 vol d^{-1} produced strong fluctuations in pH at densities of 2 and 4 g FW^{-1} l^{-1}, higher water exchange rates (12 vol d^{-1}) had a stabilizing effect on pH at all densities (Fig. 1B) and on temperature.

Ammonia levels in the fishpond effluent showed marked daily oscillations, with maximum concentrations during the "dark" period and minimum during the "light" period (Fig. 1A). Daily NH_4^+ flow was quite stable during the experimental period. NH_4^+ losses due to the system (by water recirculation and aeration of tanks without seaweed) were insignificant (less than 1.5%).

NUE were inversely related to water exchange rates and directly related with stocking density (Table 1). However NUR were directly related to

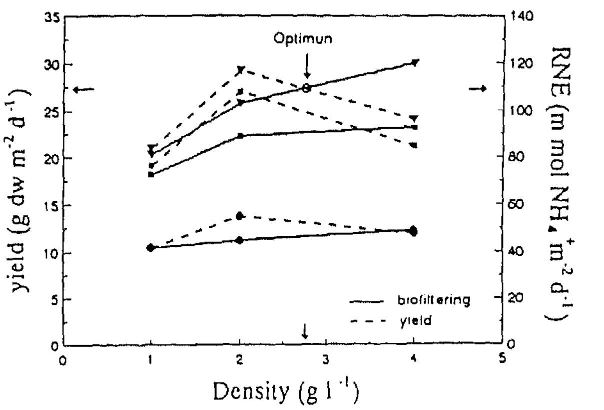

FIG. 4.- Yield (discontinuous line) and NUR (continuous line) at the three densities (1, 2 and 4 g l^{-1}) and water exchange rates of 4 (●), 8 (■) and 12 (▲) vol d^{-1}.

both flow rate and stocking densities (Table I). NUE during the light period were higher than during the dark period (Table 1). However, non significant differences in NUR ($p > 0.05$ ANOVA) were detected between light and dark periods at lowest water exchange rates (4 and 8 vol d^{-1}) (Fig.2). At higher ammonia flows (achieved by higher water exchange rates) NUR were higher during the light period at all densities ($p < 0.05$ ANOVA) (Fig.2).

NUR per unit of biomass was inversely related to density, both in dark and light periods (Fig. 3). Nitrogen uptake capacity by *Ulva rigida* plants in the light period seems to become saturated at NH_4^+ flow rates of 18.8 µmol NH_4^+ g FW^{-1} h^{-1} . However, during the dark period plants are nearly saturated at 11.8 µmol NH_4^+ g FW^{-1} h^{-1} (Fig.3). Increments in yield shows a very high correlation ($\alpha = 0.999$) with increments in NUR per unit of biomass per day.

DISCUSSION

Daily ammonia oscillations are due to the fish feeding period (between 9:00 and 14:00 h) which produce a peak of ammonia 7 hours later (Fig. 1A) due to fish excretion and microbial degradation of leftover food. Differences in the timing of peak ammonia levels reported by PORTER *et al.*, (1987) (4.5 hours after feeding) might be related to differences in fish age and feed.

The decrease in growth rate by the increase in density in our cultures is probably due to self-shading according to DUKE *et al.*, (1986); (1989a); LAPOINTE and TENORE, (1981), but may also depend on carbon limitation according to DEBUSK *et al.*, (1986).

Even though inorganic carbon concentrations in wastewater inflow have been increased by heterotrophic breakdown of dissolved organic substances (KROM and NEORI, 1989) and fish respiration (PIEDRAHITA, 1990) which results in a decrese of the pH, our results indicate that cultures over 2 g FW l^{-1} (0.86 kg m^{-2}) might be carbon limited (Fig. 1B). At 15:00 hours, the rise in pH, especially with lower exchange rate (4 vol d^{-1}) indicate that the buffering capacity of the carbonate system is decreasing, and photosynthesis causes the pH rise. According to VANDERMEULEN and GORDIN (1990), the higher pH values recorded under such conditions would indicate carbon limitation. Moreover, yield doubling (at the three densities), when doubling (8 vol d^{-1}) or tripling (12 vol d^{-1}) exchange rates (Table 1), fits well with similar increases in the amount of carbon provided to the plants per unit of time. The similar pH values of 1 g FW l^{-1} at 4 water exchanges per day and 4 g FW l^{-1} at 12 vol d^{-1} (Fig. 1) shows that photosynthetically assimilable carbon can be maintained at the same levels at four fold higher densities by a three fold increase in flow rate, which in tum produces almost 2.5 times more biomass and 3 times more NUR (Fig.3). However, NUE are lower than at 1 g FW l^{-1} and 4 vol d^{-1} (Table 1).

NUE are similar to those described by VANDERMEULEN and GORDIN (1990) and COHEN and NEORI (1991) for *Ulva lactuca*. The higher yields and NUR obtained by COHEN and NEORI (1991) at similar ammonia fluxes probably depends on the higher (almost double) irradiation in their experiments. Regarding the variable to maximize, efforts to maximize NUE and NUR values in biofiltering experiments resemble growth rate and yield values applied to production. Considerations based on only NUE will lead to wrong conclusions on its application as a biofilter (as growth rate on biomass production) (Figs. 2 and 3).

The maximum yields obtained at 2 g FW l^{-1} (0.86 kg FW m^{-2}) at any exchange rate, are in accordance with the optimum density to maximize production in *Ulva* described by LAPOINTE and TENORE (1981) and RYTHER *et al.*,(1984). The absence of correlation between yield and NUR might be explained by: i) a higher excretion of organic matter promoted by the stress (HELLEBUST, 1974) of highly dense cultures, or ii) a lower C:N ratio of the biomass at highly dense cultures (as reported by DUKE *et al.*, 1989b; VANDERMEULEN and GORDIN, 1990; COHEN and NEORI, 1991). From these results, and in accordance with DUKE *et al.* (1986; 1989a) it seems evident that ammonium uptake by *Ulva* is less limited by light than growth. However, the conclusion is just the opposite when analysing the data per unit of biomass in light and dark periods (discussed below).

Contrary to results obtained by VANDERMEULEN and GORDIN (1990) (same NUE during day and night periods) our data shows differences in NUE during light and dark periods (Table 1). These apparent contradictory results might be explained by the low ammonia flow in the experiments of those authors. In agreement with the data reported by COHEN and NEORI (1991) with *Ulva lactuca*, differences in NUE during light and dark periods are shown at high ammonia fluxes.

Higher NUR during dark period at low water exchange rates (4 vol d^{-1}) (Fig. 2) might be explained by the lower NH_4^+ fluxes during the light period. Higher NUR in light at the highest water exchange rates (12 vol d^{-1}) at all densities (Fig. 2) can be explained by the increase in ammonia (achieved by higher water exchange rates) and inorganic carbon flows. Similarities of NUR (and NUR per unit of biomass) in the dark at 12 and 8 vol d^{-1} (Figs. 2 and 3) indicate that dark cultures are N-saturated at 8 exchange rates (107 mmol m^{-2} d^{-1}), but not light cultures at any density. From Fig. 3, it seems that N-saturating flow rate in light lies around 18.8 µmol NH_4^+ g FW^{-1} h^{-1}. As the N-saturating threshold flow per unit of biomass varies significatively between the three densities (higher uptakes in plants grown at lower densities = lower selfshading) (Fig. 3), clearly shows that light has a strong influence in N-efficiency of the plant, masked by opposite efficiencies of the system (Table 1).

HARLIN *et al.* (1979) used *Gracilaria* sp to remove the ammonium produced by the fish *Fundulus heteroclitus*, removing lightly more ammonium (30 - 54 mmol kg^{-1} FW d^{-1}) than was produced by an equal biomass of fish. Similar results were reported by HAGLUND and PEDERSEN (1993) in a co-culture system of *Gracilaria teniustipitata* Zhang et Xia with rainbow trout. Daily NH_4^+ uptake rates of 40 mmol kg^{-1} FW d^{-1} were obtained at a seaweed:fish biomass ratio of 1:1. Our results, indicate that at least two times higher (91.6 mmol kg^{-1} FW d^{-1}) uptake rate efficiencies than that of *Gracilaria teniustipitata* can be obtained at four times higher fish concentration.

To maximize biofiltering efficiency of the waste-water from *Sparus aurata* hatcheries and seaweed yield, the fish should be feed as early in the morning as possible (in order to provide maximum NH_4^+ concentrations during the light period), the density of seaweeds should be adjusted to 2.7 g FW l^{-1} (1.2 kg FW m^{-2}) and water exchange rates should be (at least) 12 vol d^{-1} (150 mmol m^{-2} d^{-1}). Such conditions (at a seaweed:fish biomass ratio of 1:4 in our experimental system), will yield 27 g DW m^{-2} d^{-1} and a daily ammonia uptake rate of 110 mmol m^{-2} d^{-1} (76 % of the daily input at 12 vol d^{-1}).

ACKNOWLEDGMENTS

We wish to thank Marianne Pedersén (Dept. of Physiological Botany, University of Uppsala, Sweden) for critical reading of the manuscript. Pipo Femández-Palacios Barber and his group (Sección de Cultivos, Instituto Canario de Ciencias Marinas) are also gratefully acknowledged for their valuable colaboration. This work was partially supported by CICYT (MAR-91-1237) and a grant of the University of Las Palmas G.C. to M. Jiménez del Río.

REFERENCES

COHEN I. and A. NEORI.- 1991. *Ulva lactuca* biofilters for marine fishponds effluents. *Bot. mar.*, 34: 475-482.

DEBOER J. and J. RYTHER.- 1977. Potential yields from a waste-recycling algal mariculture system. *In*: R.W. KRAUSS (ed.): *The Marine Plant Biomass of the Pacific Northwest Coast*, pp 231-248. Oregon State University Press, Oregon, USA.

DEBUSK T.A., M. BLAKESLEE and J.H. RYTHER.- 1986. Studies on the outdoor cultivation of *Ulva lactuca*. *Bot. mar.*, 34: 381-386.

D'ELIA C.F. and J.A. DEBOER.- 1978. Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *J. Phycol.*, 14: 266-272.

DUKE C.S., B.E. LAPOINTE and J. RAMUS.- 1986. Effects of light on growth, RuBPase activity and chemical composition of *Ulva* species (Chlorophyta). *J. Phycol.*, 22: 362-370.

DUKE C.S., W. LITAKER and J. RAMUS.- 1989a. Effect of the temperature, nitrogen supply and nitrogen tissue on ammonium uptake rates of the Chlorophyte seaweeds *Ulva curvata* and *Codium decorticulatum*. *J. Phycol.*, 25: 113-120.

DUKE C.S., W. LITAKER and J. RAMUS.- 1989b. Effect of temperature on nitrogen limited growth rate and chemical composition of *Ulva curvata* (Ulvales: Chlorophyta). *Mar. Biol.*, 100: 143-150.

FRALICK R.A.- 1979. The growth of commercially useful seaweeds in a nutrient enriched multipurpose aquaculture system. *In*: JENSEN A. and J.R. STEIN (eds): *Proc. I Seaweed Symp., IX*, pp 692-698.

HAGLUND K. and M. PEDERSEN.- 1993. Outdoor pond cultivation of the subtropical marine red alga *Gracilaria tenuistipitata* in brackish water in Sweden. Growth, nutrients uptake, co-cultivation with rainbow trout and epiphyte control. *J. of Appl. Phycol.*, 5: 271-284

HARLIN M. M., B. THORNE-MILLER and B.G. THURSBY.- 1979. Ammonium uptake by *Gracilaria sp.* (Florideophyceae) and

Ulva lactuca (Chlorophyceae) in closed system fish culture. *In*: JENSEN A., STEIN J.R. (eds): *Proc. I. Seaweed Symp., IX*, pp 285-293.

HELLEBUST J.A.- 1974. Extracellular products. *In*: W.D.P. STEWART (ed): *Algae Physiology and Biochemistry*, pp. 838-863.

KROM M.D. and A. NEORI.- 1989. A total nutrient budget for an experimental intensive fishpond with circularly moving seawater. *Aquaculture*, 83: 345-358.

KROM M.D., A. NEORI and J. VAN RIJN.- 1989. Importance of water flow rate in controlling water quality processes in marine and freshwater fish ponds. *Bamidgeh*, 41: 23-33.

KROM M.D., C. PORTER and M. GORDIN.- 1985a. Nutrient budget of a marine fish pond in Eilat, Israel. *Aquaculture*, 51: 65-80.

KROM, M.D., C. PORTER C and H. GORDIN.- 1985b. Description of the water quality conditions in a semi-intensively cultured marine fish pond in Eliat, Israel. *Aquaculture*, 49: 141-157.

KROM M.D. and J. VAN RIJN.- 1989. Water quality processes in fish culture systems: processes, problems, and possible solutions. *In*: N. DE PAUW, E. JASPERS, H. ACKEFORS and N. WILKINS (eds.): *Aquaculture, A Biotechnology in Progress*, pp. 1091-1111. European Aquaculture Society, Berdene.

LAPOINTE B.E. and J.H. RYTHER.- 1978. Some aspects of the growth and yield of *Gracilaria tikvahiae* in culture. *Aquaculture*, 15: 185-193.

LAPOINTE B.E. and K.R. TENORE.- 1981. Experimental outdoor studies with *Ulva fasciata* Delile. Interaction of light and nitrogen on nutrient uptake, growth and biochemical composition. *J. exp. mar. Biol. Ecol.*, 53: 135-152.

NEORI A., I. COHEN and H. GORDIN.- 1991. *Ulva lactuca* biofilters for marine fishpond effluents. II. Growth rate, yield and C : N ratio. *Bot. Mar.*, 34: 483-489.

NEORI A., M.D. KROM, I. COHEN and H. GORDIN.- 1989. Water quality conditions and particulate chlorophyll a of new intensive seawater fishponds in Eliat, Israel: Daily and die variations. *Aquaculture*, 80: 63-78.

PIEDRAHITA R.H.- 1990. Detritus-based aquaculture systems. *Food Reviews International*, 6 (3): 317-331.

PORTER C.B., M. KROM, D. ROBBINS, M.G.L. BRICKELL and A. DAVIDSON.- 1987. Ammonia excretion and total N budget for gilthead seabream (*Sparus aurata*) and its effects on water quality conditions. *Aquaculture*, 66: 287-297.

RONNESTAD I. and H. KNUTSEN.- 1991. Flow injection analysis in marine aquacultural research: Ammonia excretion in marine fish larvae. *In Focus*, 14, 1: 6-8.

RYTHER J.H., T.A. DEBUSK and M. BLAKESKEE.- 1984. Cultivation and conversion of marine macroalgae. Final Subcontract Report to SERI, the U. S. Dept. of Energy, Publication SERI/STR-231-2360 DE 84004522, 81p.

VANDERMEULEN H. and H. GORDIN.- 1990. Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: mass culture and treatment of effluent. *J. App. Phycol.*, 2: 363-374.

Scient. ed.: S. Zanuy.