Aquaculture 312 (2011) 77–87

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

IMTA with *Gracilaria vermiculophylla*: Productivity and nutrient removal performance of the seaweed in a land-based pilot scale system

Maria H. Abreu [a,*], Rui Pereira [a], Charles Yarish [c], Alejandro H. Buschmann [b], Isabel Sousa-Pinto [a]

[a] CIMAR/CIIMAR – Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas, 289, 4050-123 Porto, Portugal & Faculty of Sciences, University of Porto, Portugal
[b] Centro i-mar, Universidad de los Lagos, Casilla 557, Puerto Montt, Chile
[c] Dept. of Ecology and Evolutionary Biology, University of Connecticut, 1 University Place, Stamford, CT, USA

ARTICLE INFO

Article history:
Received 12 August 2010
Received in revised form 15 December 2010
Accepted 16 December 2010
Available online 8 January 2011

Keywords:
Gracilaria
IMTA
Bioremediation
Seaweed
Productivity
Nitrogen
Carbon
Nutrients

ABSTRACT

The use of ecological engineering tools for the development of a more sustainable aquaculture is crucial. In this context, seaweed based Integrated Multi-Trophic Aquaculture (IMTA) systems are being designed to mitigate the environmental problems caused by several forms of fed aquaculture. Several macroalgal species, namely some from the genus *Gracilaria*, have been shown to be efficient biofilters,. *Gracilaria vermiculophylla* thrives in Ria de Aveiro lagoon, Portugal (40°38N, 8°43°W). It has been an unexploited resource for the production of agar. A seaweed cultivation system with 1200 L tanks was installed at a sole and turbot land-based aquaculture facility to evaluate the potential of this species as the biofilter component of an IMTA system. A year round, full factorial experiment was done, testing for the influence of stocking density (3, 5 and 7 kg m^{-2} (fw)), water exchange rate (100 and 200 L h^{-1}) and time of the year on *G. vermiculophylla*'s relative growth rates (RGR), productivity and nutrient removal.

G. vermiculophylla was able to maintain a good overall performance; however, results indicate that the culture conditions require adaptations throughout the year in order to attain successful productivities. In general, biomass production and nutrient removal were negatively related to the cultivation densities in the system. In the tanks seeded with 3 kg fw m^{-2}, the production of *G. vermiculophylla* was 0.7 ± 0.05 kg dw m^{-2} $month^{-1}$; this biomass removed 221 ± 12.82 g m^{-2} $month^{-1}$ of carbon and 40.54 ± 2.02 g m^{-2} $month^{-1}$ of nitrogen ($\pm 0.03\%$ of the monthly fish N inputs). Temperature and light were the main environmental factors conditioning the growth and nutrient removal performance of the seaweed. With the appropriate upscaling, this pilot IMTA system is ready for implementation at fish aquaculture operations. *G. vermiculophylla* has proved to be an efficient component of land-based IMTA systems with environmental and potentially economic benefits for the fish farm.

1. Introduction

Globally, aquaculture continues to be the fastest growing animal food-producing sector. In 2006 it had already equalled wild fisheries in the world's fish supply. Nonetheless, growth rates for aquaculture production are beginning to slow down (FAO, 2009), partially due to the increasing public concerns about aquaculture practices and fish quality. Worries about genetic modified organisms, sanitary issues but also the perception that aquaculture can harm the environment has lead to a rather negative social image of the aquaculture industry (FAO, 2009). In fact, in water downstream from aquaculture farms, changes in the levels of oxygen, suspended organic matter, inorganic nutrient, heavy metals and even drugs can be measured (Buschmann et al., 2008a; De Casabianca et al., 1997; Mendiguchía et al., 2006; Mente et al., 2006; Sanderson et al., 2008). These may negatively impact the downstream biological communities (e.g. Holmer et al., 2008).

The use of ecological engineering tools, such as Integrated Multitrophic Aquaculture (IMTA), to convert monoculture into an ecological and thus more sustainable aquaculture is crucial (Buschmann et al., 2008a; Chopin et al., 2008; Costa-Pierce, 2010; Naylor et al., 2000). In IMTA, seaweeds assimilate the fish-excreted ammonia, phosphate and CO_2, converting them into potentially valuable biomass. With this treatment, effluents can recirculate back to the fish ponds or be discharged without endangering the environment (Chopin et al., 2001; Neori et al., 2004). The advantages of using seaweeds as the biofilter component of the IMTA systems came to light more than 30 years ago (Ryther et al., 1975) but are now becoming widely accepted (Hayashi et al., 2008; Neori et al., 2007; Ridler et al., 2007).

Thus, IMTA arises as a sustainable approach, with positive environmental and socio-economic benefits for the fed aquaculture industry (FAO, 2009; Nobre et al., 2010; Ridler et al., 2007). Its application in land-based intensive fish farms can also be a tool to increase recirculation practices and establish full recirculation

* Corresponding author. Tel.: +351 223401835.
E-mail address: mhabreu@ciimar.up.pt (M.H. Abreu).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.12.036

aquaculture systems (RAS) with all its known associated benefits (Piedrahita, 2003; Wik et al., 2009). Moreover, regulations and incentives on this matter are slowly coming into effect, for example, in Europe (European Regulation N°710/2009) and USA (http://seagrant.gso.uri.edu/sustainable_seafood/ecolabeling.html).

The economic value of the biomass, besides its biofiltration efficacy, is a consideration in choosing the seaweed species to work with in IMTA systems. The cultivation of seaweeds in IMTA promotes higher productivity levels and with less variability than natural seaweed beds due to higher and more constant nutrients availability (Abreu et al., 2009; Lüning and Pang, 2003). Good examples of significant revenues for the fish aquaculture when adopting the IMTA approach already exist with *Gracilaria* (Abreu et al., 2009; Buschmann et al., 2001; Neori et al., 2000), *Ulva* (Bolton et al., 2009) and kelp species (Buschmann et al., 2010; Chopin et al., 2008).

Gracilaria is one of the world's most cultivated and valuable seaweeds (Buschmann et al., 2008b; Oliveira et al., 2000; Yarish and Pereira, 2008). Traditionally, its economic importance comes from the phycocolloid industry, being the main source of agar (Armisen, 1995; Peng et al., 2009). Several studies have successfully used *Gracilaria* species as biofilters. These were mostly developed in tanks (Buschmann et al., 1994, 1996; Chow et al., 2001; Friedlander and Levy, 1995; Hanisak, 1987; Martinez-Áragon et al., 2002; Matos et al., 2006; Neori et al., 1998, 2000), offshore systems (Abreu et al., 2009; Anderson et al., 1999; Buschmann et al., 2008c; Halling et al., 2005; Troell et al., 1997) and to a lesser extent in ponds (Haglund and Pedersen, 1993; Jones et al., 2002; Marinho-Soriano et al., 2002, 2009; Nelson et al., 2001; Shpigel and Neori, 1996). The majority of the studies achieved good results on productivity and agar content, the main destiny for the biomass. Changes in the amount and quality of this phycocolloid in algae produced with distinct nutrient conditions are also well documented (Martinez and Buschmann, 1996; Troell et al., 1997; Friedlander, 2001).

Gracilaria vermiculophylla thrives in Ria de Aveiro lagoon, Portugal (40°38N, 8°43°W). This taxonomic classification was molecularly confirmed by Saunders (2009); until then, this species was misidentified as *G. verrucosa* and *G. bursa-pastoris* (e. g. Matos et al., 2006). It is currently the dominant species at that site (Silva et al., 2004), being present throughout the year (Abreu et al., 2010). *G. vermiculophylla* may have been exploited at this lagoon since the 70s as a component of the vegetable mixture ("moliço") which was used as a soil conditioner (Silva, 1985).

G. vermiculophylla withstands a wide range of environmental parameters and is now cosmopolitan in its occurrence (Nyberg and Wallentinus, 2009). Controlled experiments with the species revealed similar growth rates at a broad range of temperature and light conditions with no differences between the different life stages. Laboratory nitrogen uptake studies revealed high N uptake values and a preference for ammonia N sources (Abreu et al., 2010). A small scale IMTA cascade system testing the biofiltration efficiency of several macroalgae species (Matos et al., 2006) provided results indicative of a good performance of *G. vermiculophylla* (misidentified in that study as *G. bursa-pastoris*). Moreover, this species produces food grade agar (Villanueva et al., 2009) and can be used as animal protein replacement in fish feed (Matos et al., 2006; Pereira et al., 2010). All these facts make *G. vermiculophylla* a potential good candidate for the biofilter component of an IMTA system.

A review by Troell et al. (2003), listed some of the main lacks in IMTA research. These include studies at relevant scales for extrapolation or commercial implementation; experimental designs to facilitate statistical analyses and comparisons; systems designed to couple nutrient removal efficiency plus high seaweed productivity and long term studies to evaluate the temporal variability of the systems.

In this study, we hypothesized that the productivity and consequent nutrient removal performance of the seaweed biofilter are affected by its cultivation density and the water flow (e.g. nutrient load) of the system. It was expected that the optimal combination of these factors would change throughout time due to the seasonal variation of environmental parameters. The scale under which this study was conducted was expected to provide information necessary for the implementation of a commercial scale system of this kind.

2. Methodology

2.1. The cultivation system

The *G. vermiculophylla* cultivation system was established at a land-based fish commercial intensive aquaculture farm (A. Coelho & Castro, Lda) in northern Portugal. This facility produces annually around 40 t of turbot (*Scophthalmus rhombus* (Linnaeus, 1758), 5 t of sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and 500 000 Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858). Recently, the company has been moving towards a full recirculation aquaculture system (RAS), thus its collaboration in this study.

Twelve 1200 L (foot print of 1.5 m^2 each) white polyethylene tanks (light transparency ≈ 10%) were set to receive independent flows of water (mix of fish effluent with clean seawater) and were used to grow the seaweed (Fig. 1). The mean concentration of the nutrients in that water was 7.2 mg L^{-1} (nitrate), 2.2 mg L^{-1} (total ammonium) and 1.8 mg L^{-1} (orto-phosphate), during the 9 months that lasted the experiment. The water flow was adjusted manually for each tank and the seaweeds were kept in constant movement by air diffusers placed in the bottom of the tanks. This helped to maximize the seaweeds' exposition to light and nutrients in the water. The cleaner effluent from the seaweed tanks was re-introduced into the fish water system.

2.2. Environmental parameters

Water temperature was monitored throughout the study. For each water flow condition, 2 sensors (ONSET, Tidbit, USA) were set to continuous register the average hourly temperature. The incident sunlight was also monitored. Every week, through day cycles measurements, the number of light hours and the irradiance was registered with a spherical light sensor (Spherical Quantum Scalar Sensor Mod. QSL – 2100 Biospherical Instruments – Inc, USA). These measures were always associated with photosynthesis' monitoring

Fig. 1. System for seaweed cultivation. 12 white polyethylene tanks (1200 L; 1.5 m^2). These were continuously supplied with the fish effluents and the outflow was recirculated back to the fish tanks. In detail, *Gracilaria vermiculophylla* biomass exhibiting good coloration and with no epiphytes.

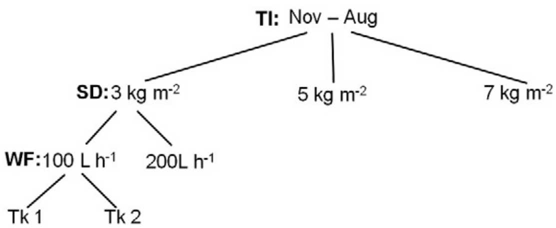

Fig. 2. Experimental design applied in the land based IMTA experiment with *Gracilaria vermiculophylla*. The influence of *time* (Ti — 9 levels), *stocking density* (SD — 3 levels) and *water flow* (WF — 2 levels) in the growth and nutrient removal performance of the seaweed was measured in 2 replicate tanks (TK) per condition.

(maximal photosynthetic efficiency) and thus always taken on sunny days. Salinity was occasionally measured, especially on rainy days, with a multi-parametric sensor (multi 340i/set, WTW, Germany). Once a week, the pH water levels were monitored in different times of the day (1: dawn; 2: morning; 3: noon; 4: pos-sunset) using a portable pH meter (PHC101, HACH, USA).

2.3. Experimental design

A preliminary experiment was done to determine the best stocking densities of *G. vermiculophylla* to be used in this study. Twelve stocking densities, from 1 to 9 kg m^{-2} (fresh weight) were tested under a water flow of 100 L h^{-1}. After 2 weeks, a regression curve indicated that 5 kg m^{-2} was the best stocking density to attain high production levels (unpublished data); this was in accordance with previous results reported by Matos et al. (2006).

Based on these results, 3 stocking densities were used on the main experiment: 3, 5 and 7 kg m^{-2}. These were tested under two water flows: 100 L h^{-1} and 200 L h^{-1}. The experimental performance of the system throughout time was followed to evaluate the influence of the natural seasonal fluctuations of light and temperature regimes on the alga growth and nutrient removal. An orthogonal array of these 3 factors was tested on the growth parameters (Relative Growth Rates – RGR & Productivity – Biomass production per area units) and nutrient (nitrogen and carbon) removal capacity of *G. vermiculophylla*. Each treatment was replicated in two tanks (see Fig. 2).

The “seeding” of the cultivation tanks was done with *G. vermiculophylla* collected at Ria de Aveiro; in order to reduce possible differences in the initial biomass, this was always done from the same *Gracilaria* population (40°49′29.81″ N; 8°39′59.46″ W). The seaweed was collected by hand and washed with freshwater to remove the attached mud, together with other algae or animals. After this, it was distributed along the different experimental treatments. The experiment ran from October 2008 to August 2009. Each experimental period consisted of 4 weeks (1 acclimation + 3 collecting data). For each the tanks, in order to minimize any tank effect. Between each period, the tanks were emptied and cleaned.

2.4. Calculations of growth parameters

Every week, the biomass was totally removed from each tank and distributed in plastic baskets. These were allowed 20 min to drain the excess superficial water and weighted on a digital dynamometer (±20 g). After restocking the tanks with the correspondent experimental cultivation density, the produced *Gracilaria* was cleaned and part of it sub-sampled for assessment of epiphytes' proliferation. The excess was dried for following application studies.

The RGR of the seaweeds in the tanks was determined by the formula: RGR (% day^{-1}) = Ln (FW) − Ln (IW)/T*100 where FW = Final fresh weight, IW = Initial fresh weight and T = Days in culture. The productivity of the tanks was calculated by the equation: (g (dw) m^{-2} wk^{-1}) = 0.17 × [(FW − IW)/1.5 m^{-2}]; wk stands for week, 0.17 is the proportion dry weight to fresh weight (mean of 50 samples) and 1.5 m^2 is the area of the tank.

2.5. Carbon and nitrogen tissue analysis

Samples of *G. vermiculophylla* (n = 5) were collected from the same natural population used to seed the 1200 L tanks and analysed for initial tissue C and N content. After 4 weeks in culture, samples were taken from each experimental condition (2 whole thalli per tank). All the samples were oven dried (60 °C for 48 h) and maintained in a dry place until analysis. At the University of Connecticut Seaweed Marine Biotechnology Lab (UConn), they were kept in the oven (40 °C) for 24 h to eliminate any residual moisture and analysed for total C and N tissue content (% dw) using a CHNS/O Analyser (Series II, 2400 Perkin–Elmer, USA). The carbon and nitrogen removal of each experimental condition was calculated by combining productivity with the results from the tissue analysis.

2.6. Statistical analysis

The average values of RGR and productivity were calculated for each experimental period (3 weeks) and used for RGR and productivity statistical analysis.

A multi-factorial analysis of variance (ANOVA) was used to analyse the influence of time (random, 9 levels), stocking density (fixed, 3 levels) and water flow (fixed, 2 levels) on the RGR and productivity of *G. vermiculophylla*. The same was done to evaluate the influence of those factors on the carbon and nitrogen removal performance of the system. Cochran's heterogeneity of variances test was applied to the data. Significant differences between treatments were analysed *a posteriori* with a Student–Newman Keuls (SNK) test ($\alpha = 0.05$). All statistical analyses were performed using the software GMAV v5 (EICC, The University of Sydney, Australia) licensed to Maria H. Abreu.

Table 1
Environmental parameters measured at the seaweed system for each experimental period — set 1. WMT: water temperature (mean ± se); Max–Min: extreme temperature values; MSI: surface irradiance (mean ± se); MI: maximal registered value of irradiance.

	Nov–Dec	Jan–Feb	Apr–May	Jun–Jul–Aug
WMT (°C)	10.9 ± 0.2	13.4 ± 0.1	15.9 ± 0.0	20.17 ± 0.0
Min–Max (°C)	7.3–5.5	10.0–17.0	10.8–22.4	15.5–26.5
MSI (μmol m−2 s−1)	1419.8 ± 34.4	1399.6 ± 51.4	1485.0 ± 18.9	1470.6 ± 18.8
MI (μmol m−2 s−1)	1963.24	1917.13	2873.1	3329.0

Table 2
Environmental parameters measured at the seaweed system for each experimental period — set 2. MHL: number of hours of light (mean ± se); RD: total number of rainy days (SNIRH — Information System of the Portuguese Hydric Resources); pH: maximal and minimal pH water levels (mean ± se) and respective experimental treatment.[a]

	Nov	Dec	Jan	Feb	Apr	May	Jun	Jul	Aug
MHL(n°)	11	10	10	11	13	15	16	16	15
RD(n°)	9	13	21	26	12	9	12	9	0
pH(max)	8.6 ± 0.1 (3_5LN)	8.3 ± 0.0 (3_3LN)	8.4 ± 0.2 (3_3LN)	8.7 ± 0.0 (3_3LN)	8.5 ± 0.1 (3_3LN)	8.9 ± 0.1 (3_5LN)	8.7 ± 0.0 (3_5LN)	8.8 ± 0.1 (3_3LN)	8.7 ± 0.1 (3_3LN)
pH(min)	7.7 ± 0.0 (1_3.HN)	7.4 ± 0.0 (1_5.HN)	7.5 ± 0.0 (1_5.HN)	7.3 ± 0.1 (1_3.HN)	7.4 ± 0.0 (1_5.HN)	7.5 ± 0.0 (1_7.HN)	7.3 ± 0.0 (1_5.HN)	7.3 ± 0.0 (1_7.HN)	7.2 ± 0.0 (1_5.HN)

[a] Experimental treatments are between brackets (daytime_stockingdensity.waterflow). Daytime: 1 = dawn, 2 = morning, 3 = noon, 4 = sunset; stocking density (kgm-2): 3, 5 and 7; water flow ($l\ h^{-1}$): HN = 200 and LN = 100.

3. Results

3.1. Environmental parameters

The experimental periods differentiated the most in terms of temperature and number of hours with light (see Tables 1 and 2). The variation reflected the season's fluctuation, as expected for the Northern Hemisphere. Mean temperatures oscillated between 10.96 ± 0.19 °C and 20.17 ± 0.03 °C, with no differences for the two water flows. Extreme temperature values were registered in December (7.3 °C) and July (26.5 °C).

The daily mean irradiance reaching the surface of the tanks in sunny days (see Methodology section) was similar throughout the experiment (1400 μmol $m^{-2}\ s^{-1}$), although with highest maximal values in spring and summer (~3000 μmol $m^{-2}\ s^{-1}$) (Table 1). It is important to notice the amount of rainy days during the experiment (Table 2). The precipitation followed the general trend for Portugal, except for the periods of June and beginning of July, which were exceptionally wet for normal summer time values. Nonetheless, even after long periods of rain, the salinity inside the tanks never fell below 30 ppm.

The average pH of the water levels oscillated between 7.2 and 8.9 (Table 2). The interaction between water flow and the daily time of

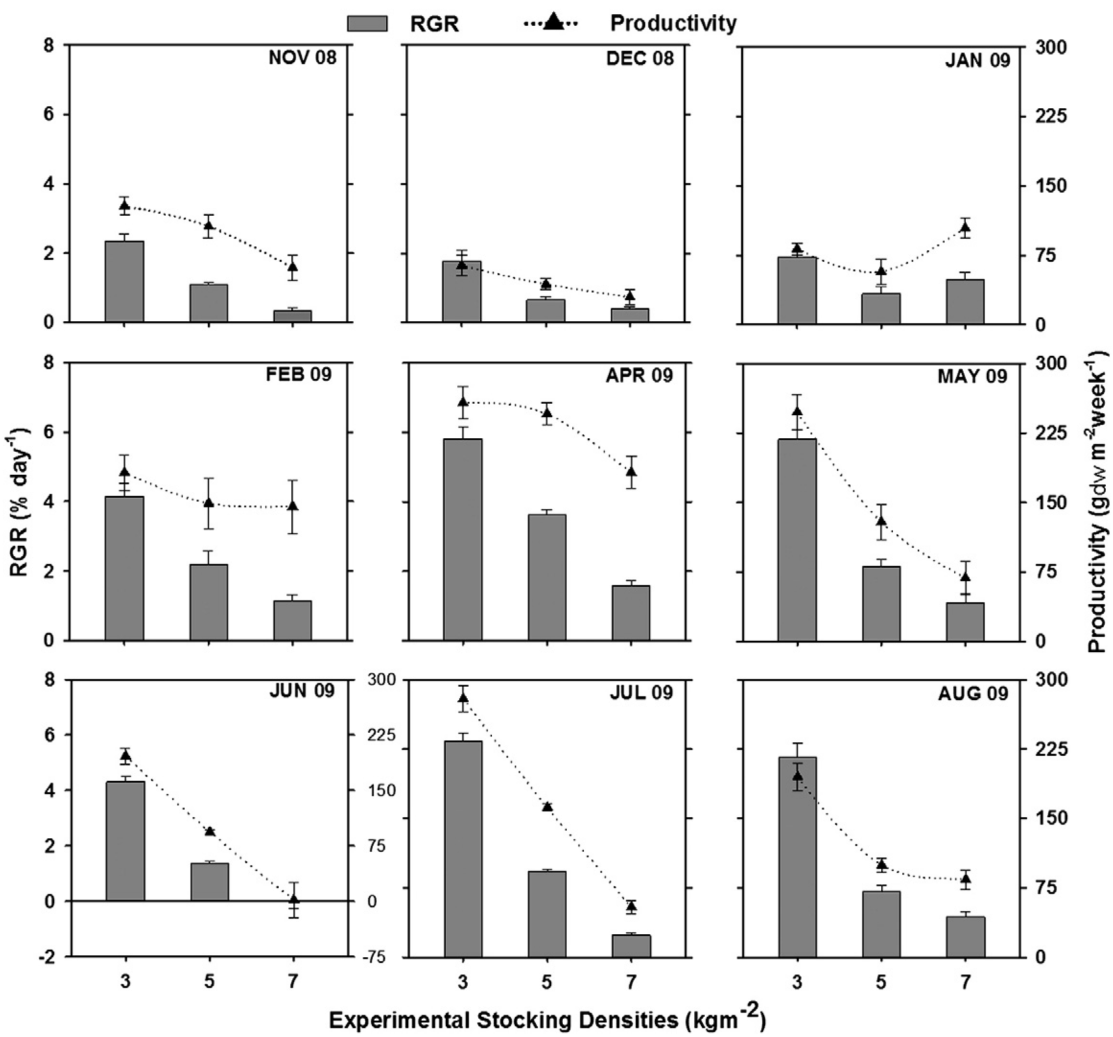

Fig. 3. Relative growth rate (mean ± SE, n = 4; gray bars) and productivity (mean ± SE, n = 4; dotted line) of *Gracilaria vermiculophylla* cultivated under 3 stocking densities (3, 5 and 7 kg m^{-2}) in 1200 L tanks receiving nutrient enriched water from a commercial fish aquaculture.

Table 3
ANOVA testing the effects of time (Ti), stocking density (SD) and water flow (WF) on the RGR, productivity and carbon and nitrogen removal performance of the seaweed.[a]

	RGR			Productivity		N removal			C removal	
Source of variation	df	MS	F	MS	F	df	MS	F	MS	F
Ti	8	11.2	59.4*	3.7E+04	42.8*	7	3015.7	64.0*	1.2E+05	105.1*
SD	2	109.5	34.2*	8.8E+04	11.9*	2	4881.6	7.5*	1.7E+05	7.9*
WF						1	383.6	3.4*	445.9	0.1
Ti×SD	16	3.2	17.0*	7.4E+03	8.4*	14	651.9	13.8*	21723.6	18.5*
Ti×WF						7	113.6	2.4*	3353.6	2.9*
SD×WF						2	4.9	0.1	928.1	0.4
TI×SD×WF						14	90.8	1.9*	2460.3	2.1*
Residuals	81	0.2		8.7E+02		144	47.1		1172.6	
Cochran's test	C=0.1			C=0.1		C=0.1			C=0.1	
Transformation	None			None		None			None	

[a] The (*) stands for significant differences found between treatments.

measure (1: dawn; 2: morning; 3: noon; 4: post-sunset) had a significant effect on the pH water levels ($P<0.05$) in all the experimental periods. The lowest values were registered before sunrise, and at the highest water flow. Maximal pH values (were reached in the tanks receiving the lowest water flow (100 L h^{-1}), at 13:00 (solar peak time = maximal photosynthetic rate) and always at the lowest density (3 kg m^{-2}). Although with no statistical difference, it was possible to perceive a pattern of lower pH with increasing stocking densities during the warmer periods.

3.2. Growth performance

G. vermiculophylla was able to grow in the IMTA system throughout the entire course of the experiment and under all conditions tested, except for June 2009 at 7 kg m^{-2}, when negative growth values were registered (Fig. 3). The ANOVA performed on the 3 factors, revealed that the water flow had no significant effect on either RGR ($P>0.9$) or productivity ($P>0.4$). Therefore, this factor was pooled and a new ANOVA was done considering only factors time and

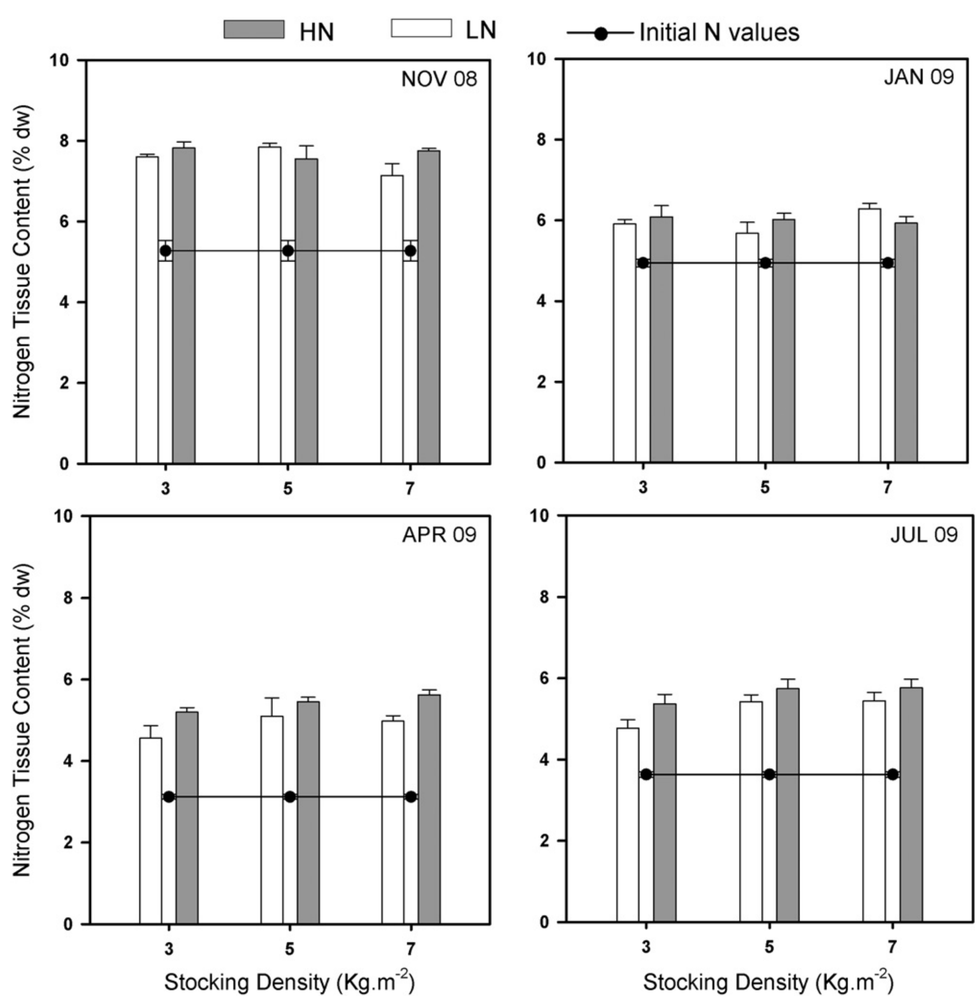

Fig. 4. Mean (± SE; n = 5) nitrogen concentration (% dw) in tissues of *Gracilaria vermiculophylla* from the natural population and after 4 weeks growing in the IMTA system under three stocking densities (3, 5 and 7 kg m^{-2}) and 2 water flows (HN = 200 L h^{-1}, gray bars and LN = 100 L h^{-1}, white bars).

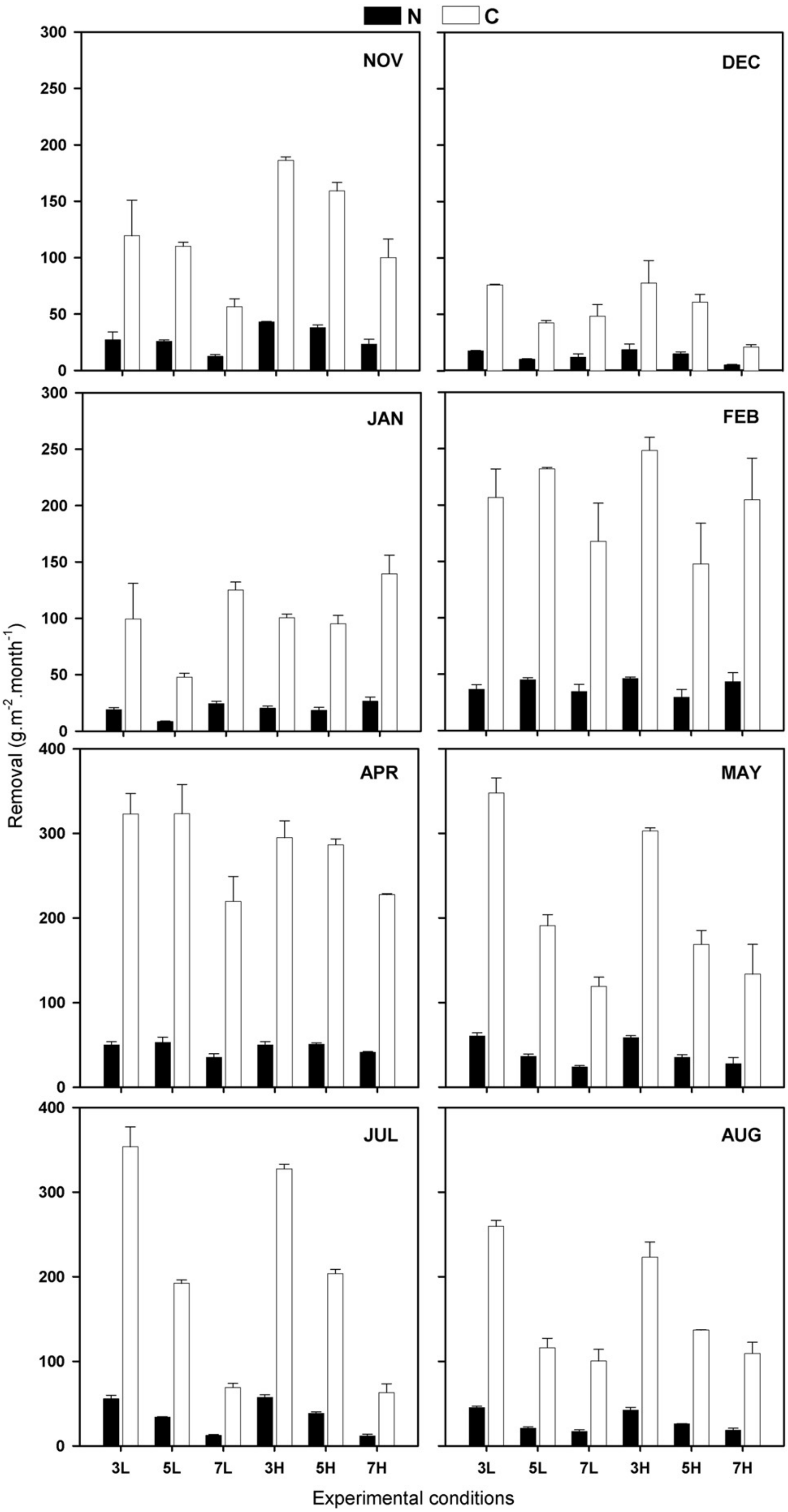

Fig. 5. Mean (±SE; n = 4) carbon (white bars) and nitrogen (black bars) removal rate (g m^{-2} $month^{-1}$) for the entire course of the experiment. Representation of each combined set of stocking densities (3, 5 and 7 kg m^{-2}) and water flows (H = 200 L $h^{-1}$and L = 100 L h^{-1}).

stocking density. The interaction between these two factors caused the significant differences found for RGR and productivity (Table 3).

RGR was lower with increasing stocking density except for the winter months of December and January (SNK: 5 kg m^{-2} = 7 m^{-2} < 3 m^{-2}; Fig. 3 — gray bars). The highest RGR value (6.23% ± 0.25) was observed in July for *Gracilaria* cultivated under a density of 3 kg m^{-2}. The lowest value (no growth at all) occurred in June in the tanks stocked with 7 kg m^{-2} (Fig. 3). However, considering the abnormal climatic conditions observed at that time, one can instead consider the minimal values occurring in November 08 (0.33% ± 0.08), under the same stocking density condition.

The "seasonal" effect on *Gracilaria* growth was more evident in the 3 kg m^{-2} condition, with 3 distinct growth periods (SNK: Dec = Jan = Nov < Feb = Jun < Apr = May = Jul = Aug). During the coldest and less luminous months, the minimal RGR values of 1.78% ± 0.30 were registered; the highest value (6.23% ± 0.25) occurred with the opposite environmental conditions. At 5 kg m^{-2}, RGR was only distinct in April and for 7 kg m^{-2}, no differences were found between the experimental periods.

In terms of productivity, the results were slightly different. Although the lowest density showed the best overall performance, there were some exceptions (Fig. 3, dotted line). Under winter conditions (December through February), there were no differences in the productivity of the 3 cultivation densities, with values ranging from 27.67 ± 8.44 to 104.36 ± 11.32 g (dw) m^{-2} $week^{-1}$. In November and April, the productivity values of *Gracilaria* were lowest at the highest stocking density (59.36 ± 13.45 g (dw) m^{-2} $week^{-1}$) and similar between 3 and 5 kg m^{-2}, reaching a maximal value of 256.89 ± 17.92 g (dw) m^{-2} $week^{-1}$ during that period.

With the increase of temperature and light hours, as in RGR, the 3 cultivation densities performed significantly different (SNK: 7 kg m^{-2} < 5 kg m^{-2} < 3 kg m^{-2}). In June, the absolute values of productivity dropped (minimal: 1.98 ± 23.24 g (dw) m^{-2} $week^{-1}$), although maintaining the same pattern as May or July (Fig. 3). The overall maximal productivity was attained in July (279.37 ± 13.95 g (dw) m^{-2} $week^{-1}$, Fig. 3) in the tanks seeded with 3 kg m^{-2} and dropped again in August. Similar to the RGR results, the SNK tests revealed that the lowest stocking density (3 kg m^{-2}) was the more variable with time, having four periods of distinct productivity values (SNK: Dec = Jan < Nov < Feb = Aug = Jun < Apr = May = Jul). The productivity for 5 and 7 kg m^{-2} was more constant throughout the course of the experiment. Overall, it is possible to say that, despite the clear seasonal variability observed for the lowest stocking density (3 kg m^{-2}), in 8 of 9 months, RGR and productivity values were highest at this condition. 5 kg m^{-2} was the most stable condition, but with a significantly lower growth performance.

Throughout the entire experiment the epiphytes load in the tanks (*Ulva* spp.) was always below 2% dw of the total biomass in the tanks. Maximal values were reached during spring and summer months, coincident with the rise of temperatures and day lengths. It is important to notice that the epiphytes were mainly proliferating on the walls of the tanks and not growing on the *Gracilaria* tissue (Fig. 1 — detail).

3.3. Tissue carbon and nitrogen content and C:N ratio

The tissue nitrogen values (% dw) from the natural population of *G. vermiculophylla* oscillated between 3.12% ± 0.06 (April 09) and 5.27% ± 0.25 (November 09). The N content always increased after the 4 weeks' growth period at the IMTA system (Fig. 4). Differences from 13% (April) to 37% (July) were registered between Gracilaria wild material and IMTA produced biomass. The ANOVA used to evaluate the influence of the experiment time (November, January, April and July) and the water flow/N input, on the final N content of the seaweed showed that the two factors significantly affect it (Time: $F_{(3, 72)} = 161.17$, $P < 0.05$ and Water flow: $F_{(1, 72)} = 11.80$, $P < 0.05$). In terms of time, the pattern was coincident with the one found for the initial seeding biomass (Apr < Jul < Jan < Nov). The water flow was generally positively related to the accumulation of N in the tissue. The lowest N tissue content was registered in April (4.56% ± 0.30–3 kg m^{-2}, 100 L h^{-1}) and the highest in November (7.84% ± 0.09–5 kg m^{-2}, 100 L h^{-1}). Regarding the carbon content, although with slight increases, no major differences were found between the values of the "seeding" population and the biomass after 4 weeks in the IMTA system; these were always around 30 to 33% dw. The C:N ratio oscillated between 6.2 (November) and 9.6 (April) in the tissue of the wild *G. vermiculophylla*. After four weeks in the IMTA system, the *Gracilaria* tissue had average C:N ratios between 4.1 (Jan, highest densities) and 6.2 (April, 3 kg m^{-2}).

3.4. Carbon and nitrogen removal

The nutrient removal capacity of the *Gracilaria vermiculophylla* system was closely related to its growth performance. The major differences found for C and N removal were caused by the interaction of the 3 factors (SD, WF and Ti) in study (Table 3). It was possible to separate two different time periods for *G. vermiculophylla* nutrient removal performance (Fig. 5, see difference in Y axis scale). From November until February, *Gracilaria* removed fewer nutrients. For that period, the 3 stocking densities had similar nutrient removal values with a general positive effect of increased water flow (Fig. 5). The lowest values were observed at 7 kg m^{-2} with the higher water flow (5.18 ± 0.5 g N m^{-2} $month^{-1}$ and 21.05 ± 2.2 g C m^{-2} $month^{-1}$).

The best nutrient removal results were registered between April and August, for *G. vermiculophylla* growing at 3 kg m^{-2}, regardless of the water flow (60.4 ± 3.9 g N m^{-2} $month^{-1}$ and 347.8 ± 17.8 g C m^{-2} $month^{-1}$, Fig. 5 — Jun). With this stocking density, the one with the best growth performance, *G. vermiculophylla* had minimal nutrient removal values under the lowest water flow (17.73 ± 0.3 g N m^{-2} $month^{-1}$ and 76.08 ± 0.8 g C m^{-2} $month^{-1}$, Fig. 5 — Dec).

4. Discussion

4.1. Seaweed performance

G. vermiculophylla was able to grow and remove nutrients throughout the 9 months of the experiment in a pilot scale seaweed biofilter tank system. The best results were achieved at a stocking density of 3 kg (ww) m^{-2}. At this condition, the seaweed system produced 0.7 ± 0.05 kg (dw) m^{-2} $month^{-1}$ of *G. vermiculophylla*. The biomass produced was free of epiphytes and removed 221 ± 12.82 g m^{-2} $month^{-1}$ of C and 40.54 ± 2.02 g m^{-2} $month^{-1}$ of N.

This work was done in a system exposed to the changing weather conditions, confirming the tolerance of *G. vermiculophylla* to a wide range of environmental conditions. The capacity of *G. vermiculophylla* to sustain growth even with large temperature variations was in accordance with the laboratory studies carried out with this species (Abreu et al., 2010; Rueness, 2005; Yokoya et al., 1999). The positive relation of growth with temperature was expected and previously documented for other *Gracilaria* species (Friedlander and Levi, 1995).

Besides temperature, light played a major role in conditioning the performance of *G. vermiculophylla*. These are usually the most important environmental parameters affecting growth and nutrient uptake of macroalgae (Lobban and Harrison, 1997; Lüning, 1990). The interaction of these factors with the seaweed load in the tanks was clear and more pronounced during spring and summer months. The optimal light and temperature conditions triggered an increase in the growth performance of *Gracilaria* cultured at the lowest densities. In the tanks "seeded" with 7 kg of *Gracilaria*, less than 15% of the incident solar irradiance reached a depth of 15 cm in the water column. This probably negatively affected the photosynthetic efficiency of the seaweeds with consequent lower levels of productivity at that stocking density. The opposite effect (photoinhibition) is expected to occur in lower stocking densities (Mata et al., 2006). However, the superior performance of *G. vermiculophylla* at 3 kg m^{-2}, suggests a high resistance of this species to high irradiance levels.

The lower temperatures and light levels were probably responsible for the minor growth and nutrient removal values registered during the autumn/winter months. During that period, the water flow probably helped to balance these adverse conditions, thus explaining the slightly better growth that was observed under the high water exchange rates. A change to a cold type macroalgae species during these months might increase the efficacy of the system. However, finding another species that could significantly outperform *G. vermiculophylla* might be a logistical challenge and implicate an additional investment for the aquaculture company.

The results obtained in this work were different from those reported in Matos et al. (2006), but confirm that *G. vermiculophylla* is an optimal species to include in an IMTA system under these circumstances. The disparities found between the two works can be due to the experimental design of each study, which included a difference in the duration of the experiment (3 vs. 9 months); in our study, the best stocking density was 3 kg m^{-2} attaining higher yields than the ones reported in that work for a stocking density of 5 kg m^{-2}. On the other hand, the effects of water flow were not different enough to show differences in the growth performance of *G. vermiculophylla*; the water exchange rate, and thus nutrient availability, was only relevant during 2 of the coldest months. This is most probably due to the high N concentration (mean values referred in Section 2.1) present at the fish farm effluent during all times (unpublished data).

Relative to other *Gracilaria* species in large scale cultivation systems, the *G. vermiculophylla* produced in this IMTA system had equivalent productivity as that reported by Hanisak (1987) for *Gracilaria tikvahiae*, with an average of 25.8 ± 1.8 g (dw) m^{-2} day^{-1}. Summer productivity of *G. vermiculophylla* in our IMTA system attained mean values of 39.9 g (dw) m^{-2} day^{-1}, similar to the ones documented by Capo et al. (1999) with *G. ferox* (currently regarded as *G. cervicornis*) and Nagler et al. (2003) with *G. parvispora*. It is important to notice that this comparison does not account for the different light conditions of the tanks used during the studies.

Although depending on the physiological traits of each species, inorganic Carbon (Ci) is the first element to be depleted in tank culture systems (Craigie and Shacklock, 1995; Hanisak, 1987). *G. vermiculophylla* thrives in the intertidal of mudflats areas and is adapted to severe changes in environmental parameters that occur at those areas (Nyberg et al., 2009). One of those adaptations can be related to the capacity of overcoming possible limiting dissolved Ci conditions (Andría et al., 2001). *Gracilaria* species cultivated in seawater with high pH levels can use the ionic source of Carbon (HCO_3^-) besides the CO_2 to photosynthesize. However, in cases of extremely high pH (normally above 9.0) this capacity could be lowered, with strong impacts on the photosynthetic rates and biomass production (Zou and Gao, 2009; Zou et al., 2004,).

During this study, pH levels never went above that critical value. Water flow was determinant for maintaining the temperature in non-lethal levels for the growth of *G. vermiculophylla*, for preventing severe drops in salinity due to periods of heavy rain periods and to probably guarantee non-limiting Ci inside the tanks. The highest pH levels were registered at the lowest water flow, 100 L h^{-1} (2 tanks per day), during the periods of higher productivity. However, these never exceeded the 9.0 pH level which can be an indicator of non-limitation by Ci, as seen for other *Gracilaria* species (Israel et al., 1999; Zou et al., 2004). This is also in accordance with the fact that no significant differences were found in growth or nutrient removal between the two water flows. So, it seems that future work with this species, in this system, could adopt a water exchange rate of 4 tanks per day (=200 L h^{-1}) or higher and thus increase the total volume of biofiltration capacity. However, this could lower the efficiency in nutrient water reduction (Matos et al., 2006; Neori et al., 2004; Troell et al., 2003). The pH of the water was lowest at the highest cultivation densities tested. This means that under those conditions, the seaweeds were not Ci limited, probably because they were photosynthesizing at very low rates (as seen by the lowest growth values). This confirms that temperature and light were the main limiting factors of the seaweed growth and nutrient removal performance when cultivated at 7 kg m^{-2}.

According to Hanisak (1987), the nitrogen critical value, which is the minimum tissue N content required for maximal growth, of *G. tikvahiae* is 2%. Considering that value, the growth of *G. vermiculophylla* does not seem to be nitrogen limited at its natural environment (mean values of 4.8% dw) and even less at the IMTA system (mean values 6.0% dw). The highest water flow promoted a significant higher nitrogen and carbon accumulation in *Gracilaria* tissue, as reported by Matos et al., 2006, but only for the winter months. After that, the difference found for the productivity performance of the tested cultivation densities annulated the variations in nutrient accumulation.

The C:N ratios determined, 4.1 (wild) and 6.2 (IMTA), also confirm that *Gracilaria* was growing in N saturated conditions since these are quite below 13.5, the ratio suggested by Hanisak (1990). The C:N ratio can also provide an indication of the nutrient uptake rate of the seaweed (Hanisak, 1990). For *G. tikvahiae*, a C:N ratio below 10 does not influence the ammonia uptake rate (D'Elia and DeBoer, 1978). This relation needs to be established for *G. vermiculophylla*. Nevertheless, if the same value is applied, it suggests that the seaweed was accumulating nitrogen not necessary for growth. This luxury uptake has also been observed in other red algae species. It is stored in pigments and amino acids and can assure growth during periods of N starvation (Bird et al., 1982; Figueroa et al., 2008; Harrison and Hurd, 2001; Kim et al., 2007; Pereira et al., 2008). The fact that *G. vermiculophylla* continues to accumulate nitrogen when cultured at the IMTA system might be an indication of the improved growth conditions, like the high water movement (Hurd, 2000; Smit, 2002) and the continuous supply of ammonia.

Laboratory experiments reported the ability of *G. vermiculophylla* to remove both ammonia and nitrate from the culture media, however with a higher affinity for the ammonium N source (Abreu et al., 2010). This had already been observed for other *Gracilaria* species (D'Elia and DeBoer, 1978; Jones et al., 2001).

Therefore, the biofilter performance of *G. vermiculophylla* was expected to be high when subjected to actual values of these nitrogen sources in a commercial scale aquaculture system. The nitrogen removal results confirmed this hypothesis and preliminary results point to a TAN ($NH_4^+ + NH_3$) reduction efficiency in the order of 80% for the best set of stocking density and water flow (Abreu et al., 2010).

Considering the nitrogen values found in the tissue of the "seeding" population at Ria de Aveiro lagoon, *G. vermiculophylla* might be playing an important role as a nutrient sink in that environment. The lagoon suffers from strong anthropogenic impact (cities, tourism, boat activities, fisheries, earth pond aquaculture, etc.) and nonpoint runoffs from extensive agriculture areas. All these nutrient sources have turned this lagoon into a site experiencing euthrophication problems (Silva et al., 2002). The function of *G. vermiculophylla* as a potential "biofilter" of this ecosystem should be considered in the management of eutrophication (He et al., 2008; Jiang et al., 2010; Yang et al., 2006; Zertuche-González et al., 2009).

4.2. Nutrient removal estimations of the pilot scale system

The average crude protein content of the feed used in this fish farm was 46.3% which is equivalent to a content of 7.41% of nitrogen (crude protein = N × 6.25). Considering a Feed Conversion Rate of 1.5 (information given by the company), and the 200 kg fed daily to the fish, it is expected the daily N load in the effluent, resultant from feed wastes, to be around 4.9 kg. Without considering any seasonal changes in the fish behaviour and no effects of the recirculation system, this would mean that, per year, the aquaculture load may be 1.8 t of N.

By performing simple calculations on the seaweed's productivity and nutrient removal average results of this work we can get to the following values: Per year, our system of twelve tanks (18 m^{-2}), stocked with 3 kg (fw) of *G. vermiculophylla* per square meter could produce around 156 kg (dw) of seaweed. This biomass would remove 8.8 kg of N. So, 18 m^2 of *G. vermiculophylla* would be able to remove 0.5% of the total N load of the fish effluent. In order to remove 25%, the seaweed cultivation area would increase to 900 m^2; to attain 100% of N removal efficacy, it would go up to 0.36 ha. Before an upscaling of the system, these estimates can be improved through a correct characterization of the nutrient budgets (including all possible sources of nutrient variation) operating at the system (Neori et al., 1998).

The cost of land is probably the most severe constraint to the development of land based IMTA systems. To make it worthwhile, it is necessary to continue to explore new and profitable applications that can bring added value to the seaweed biomass produced. Moreover, space limitation can be overcome and biofiltration performance improved by establishing multi-seaweed species systems with different N uptake dynamics (Bracken and Stachowitz, 2006), cascade systems (Matos et al., 2006) and including organic extractive components like shellfish (Neori et al., 2000).

The best way to promote IMTA is to associate economic value to the environmental services provided by the seaweeds: CO_2 sequestration, cleaner effluents and good marketing of the fish production need to have a cost associated (Neori et al., 2007; Chopin et al., 2008). The environmental service provided by mussel farms is already accounted for in Sweden, through a successful nutrient trading scheme (Lindahl and Kolberg, 2006). The cost of removing 1 kg of Nitrogen is established ca. 7USD, which is about one fifth of what the municipality would have to pay for a chemical removal process (Lindahl et al., 2005). According to Chopin (2010), the cost of removing 1 t of C can be 30 USD. So, applying those values to a *G. vermiculophylla* biofilter system with the capacity to reduce 25% of the annual N wastes, the environmental service provided by the seaweed would thus be around 4300 USD. The sequestration of C could signify an income/savings of 71 USD. Moreover, the carbon removal values would reduce the ecological footprint of the company and give it the chance of entering the carbon credits' market in Europe; this is already being implemented together with the production of microalgae for biofuel (www.oilalgae.com). The same approach for seaweeds is under debate (Buschmann et al., 2010). It is clear that if aquaculture companies get forced to include environmental costs in their budget, they may get more interested in these figures.

4.3. Biomass applications

The biomass of *G. vermiculophylla* produced in the IMTA system was screened for agar quality. Besides a new extraction method developed with this material (Sousa et al., 2010), preliminary results point to increasing gel strength when compared to the one extracted from wild *G. vermiculophylla* (Villanueva et al., 2009). Further studies will help to adjust cultivation conditions in order to meet the desired agar features.

The cost of fish feed is currently one of the major constraints in aquaculture expansion (FAO, 2009). Alternative protein sources, improved buoyancy and feed formulation are approaches that have been applied to reduce costs and nutrient wastes through increased FCR (Ramseyer and Garling, 1997; Hardy and Tacon, 2002). Several terrestrial plants (e.g. soy, corn, wheat) have been used as fish meal replacement in the feeds (Kaushik et al., 2004; Silva et al., 2009). However, most of them are agricultural crops used for human consumption giving rise to increasing feed costs. The nutritional aspects of seaweeds, although already studied to some extent (Fleurence, 1999) are still overlooked by the fish feed industry. Only a few examples of research in this area can be found for fish (Soler–Vila et al., 2009; Valente et al., 2006) and shrimp (Cruz-Suárez et al., 2009; Marinho-Soriano et al., 2007). More work and trials at relevant scales are necessary. The *Gracilaria* biomass produced in our system is also being analyzed for potential ingredient in fish feed (Pereira et al., 2010) due to the high protein value (mean value of 37.5%, with highest values of 50%). Besides protein content, these seaweeds can be a source of ultraviolet-screen substances; MAAs (mycosporine like amino-acids) have been reported in *Asparagopsis armata* (Figueroa et al., 2008) and *Gracilaria conferta* (Figueroa et al., 2010). This is still a matter to be further scrutinized.

5. Final considerations

The harvest of natural *Gracilaria* beds is not enough to meet demand (Oliveira et al., 2000; FAO, 2003). Spain is a major *Gracilaria* importer since it is home to some of the biggest agar manufacturing companies. These get their raw material from neighboring Morocco but mostly from far away Chile (*pers. comm.*). *G. vermiculophylla* biomass produces an agar with somewhat higher gel strength than reported for the Morocco material (see Givernaud et al., 1999; Villanueva et al., 2009). At the same time, an increase in fish and mollusks aquaculture is expected in the Iberia Peninsula. Hence, it seems that there is an opportunity to validate the IMTA approach in Portugal, using *Gracilaria* as the biofilter component. The biomass of *G. vermiculophylla* produced at the IMTA system, revealed to be of excellent quality for some applications: good grade agar, fish feed ingredient and possibly as a source for MAAs. Ongoing interdisciplinary research is looking forward to optimize the processes for these uses and to find new possibilities of adding value to the seaweed biomass. The production of seaweeds in IMTA can also be beneficial for the health of cultured fish (Bansemir et al., 2006; Díaz-Rosales et al., 2007).

Bringing all these economic aspects together with the environmental benefits of IMTA is critical in order to make the different stakeholders involved (scientific, commercial, management, policy makers) communicate and develop real measures to turn aquaculture into a more sustainable and socially accepted industry (Costa-Pierce, 2010). A tool from the economics/management fields came to scene and can be of great help, the DPSIR framework (Drivers–Pressure–State–Impact–Response). The application of this model to our system may help to validate the advantages of adopting the IMTA approach in this kind of land based aquacultures (Nobre et al., 2010).

G. vermiculophylla exhibited a good growth and nutrient removal performance year round, although with a slight decrease during the winter months. The best set of conditions was a cultivation density of 3 kg m^{-2} and a water exchange rate of 200 L h^{-1}. Overall, we believe that this *G. vermiculophylla* cultivation system is ready for upscaling and implementation at fish aquaculture companies interested in starting an IMTA approach based on this species.

Acknowledgments

The authors acknowledge all the help given by Pedro Roque throughout the field work and truly appreciate the support given by the company A. Coelho & Castro, Lda. The authors are also grateful for the valuable comments made to the manuscript by the anonymous reviewers. Maria H. Abreu was supported by the Portuguese Foundation for Science and Technology (FCT) with a PhD scholarship (ref: SFRH/BD/21363/2005) and the program POPH/FSE. R. Pereira was also supported by a fellowship from FCT through program POCI 2010, with the support of FEDER and FSE (SFRH/BPD/36451/2007). The field work was partially funded by project IBEROMARE (POCTEP).

References

Abreu, M.H., Varela, D.A., Henriquez, L., Villarroel, A., Yarish, C., Sousa-Pinto, I., Buschmann, A.H., 2009. Traditional vs. integrated multi-trophic aquaculture of *Gracilaria chilensis* C. J. Bird, J. McLachlan & E. C. Oliveira: productivity and physiological performance. Aquaculture 293, 211–220.

Abreu, M.H., Pereira, R., Korbee, N., Figueroa, F., Buschmann, A., Yarish, C., Sousa Pinto, I., 2010. Sustainable exploration of a non-indigenous species: the case study of *Gracilaria vermiculophylla* (Ohmi) Papenfuss in Portugal. XXth International Seaweed Symposium book of abstracts, p. 43. Ensenada, Mexico.

Anderson, R.J., Smit, A.J., Levitt, G.J., 1999. Upwelling and fish-factory waste as nitrogen sources for suspended cultivation of *Gracilaria gracilis* in Saldanha Bay, South Africa. Hydrobiologia 398 (399), 455–462.

Andría, J.R., Brun, F.G., Perez-Llorens, J.L., Vergara, J.J., 2001. Acclimation responses of *Gracilaria* sp (Rhodophyta) and *Enteromorpha intestinalis* (Chlorophyta) to changes in the external inorganic carbon concentration. Botanica Marina 44, 361–370.

Armisen, R., 1995. World-wide use and importance of *Gracilaria*. Journal of Applie Phycology 7, 231–243.

Bansemir, A., Blume, M., Schroder, S., Lindequist, U., 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture 252, 79–84.

Bird, K.T., Habig, C., DeBusk, T., 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). Journal of Phycology 18, 344–348.

Bolton, J.J., Robertson-Andersson, D.V., Shuuluka, D., Kandjengo, L., 2009. Growing *Ulva* (Chlorophyta) in integrated systems as a commercial crop for abalone feed in South Africa: a SWOT analysis. Journal of Applied Phycology 21, 575–583.

Bracken, M.E.S., Stachowicz, J.J., 2006. Seaweed diversity enhances nitrogen uptake via complementary use of nitrate and ammonium. Ecology 87, 2397–2403.

Buschmann, A.H., Mora, O.A., Gómez, P., Böttger, M., Buitano, S., Retamales, C., Vergara, P.A., Gutierrez, A., 1994. *Gracilaria chilensis* outdoor tank cultivation in Chile: use of land-based salmon culture effluents. Aquaculture Engineering 13, 283–300.

Buschmann, A.H., Troell, M., Kautsky, N., Kautsky, L., 1996. Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Gracilariales, Rhodophyta). Hydrobiologia 326 (327), 75–82.

Buschmann, A.H., Correa, J., Westermeier, R., Hernández-González, M., Norambuena, R., 2001. Cultivation of red algae in Chile: a review. Aquaculture 194, 203–220.

Buschmann, A.H., Hernández-González, M.C., Aranda, C., Chopin, T., Neori, A., Halling, C., Troell, M., 2008a. Mariculture waste management. In: Jørgensen, S.E., Fath, B.D. (Eds.), Ecological Engineering. : Encyclopedia of Ecology, 5 vols. Elsevier, Oxford, pp. 2211–2217.

Buschmann, A.H., Hernández-González, Varela, D.A., 2008b. Seaweed future cultivation in Chile: perspectives and challenges. International Journal of Environment and Pollution 33, 432–456.

Buschmann, A.H., Varela, D.A., Hernández-González, M.C., Huovinen, P., 2008c. Opportunities and challenges for the development of an integrated seaweed-based aquaculture activity in Chile: determining the physiological capabilities of *Macrocystis* and *Gracilaria* as biofilters. Journal of Applied Phycology 20, 571–577.

Buschmann, A.H., Hernández-González, M.C., Flores, R., Gutiérrez, A., Varela, D., Huovinen, P., 2010. Massive kelp production in Chile: future prospects, challenges and limitations. XXth International Seaweed Symposium book of abstracts, p. 51. Ensenada, Mexico.

Capo, T., Jaramillo, J., Boyd, A., Lapointe, B., Serafe, J., 1999. Sustained high yields of *Gracilaria* (Rhodophyta) in intensive large-scale culture. Journal of Applied Phycology 11, 143–147.

Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-Gonzalez, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into marine aquaculture systems: a key toward sustainability. Journal of Phycology 37, 975–986.

Chopin, T., Robinson, S.M.C., Troell, M., Neori, A., Buschmann, A.H., Fang, J., 2008. Multi-trophic integration for sustainable marine aquaculture. In: Jørgensen, S.E., Fath, B.D. (Eds.), Ecological Engineering. : Encyclopedia of Ecology, 5 vols. Elsevier, Oxford, pp. 2463–2475.

Chopin, T., 2010. From Integrated Multi-Trophic Aquaculture (IMTA) to nutrient and carbon trading credits: recognizing and valuating the ecosystem services and benefits of the extractive components of IMTA. Book of abstracts. : Aquaculture-World Aquaculture Society.

Chow, F., Macchiavello, J., Cruz, S., Fonck, E., Olivare, J., 2001. Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariacea) as a biofilter in the depuration of effluents from tank cultures of fish, oysters and sea urchins. Journal of the World Aquaculture Society 32, 215–220.

Costa-Pierce, B., 2010. Sustainable ecological aquaculture systems: the need for a new social contract for aquaculture development. Marine Technology Society Journal 44, 88–112.

Craigie, J.S., Shacklock, P.F., 1995. Culture of Irish moss, In: Boghen, A.D. (Ed.), Cold-Water Aquaculture in Atlantic Canada, second ed. The Canadian Institute for Research on Regional Development, Moncton, pp. 365–390.

Cruz-Suárez, L.E., Tapia-Salazar, M., Nieto-Lopez, M.G., Guajardo-Barbosa, C., Ricque-Marie, D., 2009. Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds. Aquaculture Nutrition 15, 421–430.

D'Elia, C.F., DeBoer, J.A., 1978. Nutritional studies of two red algae: II. Kinetics of ammonium and nitrate uptake. Journal of Phycology 14, 266–272.

De Casabianca, M.L., Laugier, T., Marinho-Soriano, E., 1997. Seasonal changes of nutrients in water and sediment in a Mediterranean lagoon with shellfish farming activity (Thau Lagoon, France). ICES Journal of Marine Science 54, 905–916.

Diaz-Rosales, P., Felices, C., Abdala, R., Figueroa, F.L., Pinchetti, J.L.G., Morinigo, M.A., Balebona, M.C., 2007. In vitro effect of the red alga *Hydropuntia cornea* (J. Agardh) on the respiratory burst activity of sole (*Solea senegalensis*, Kaup 1858) phagocytes. Aquaculture Research 38, 1411–1418.

EU, 2009. Regulamento (CE) N. o 710/2009 da comissão de 5 de Agosto de 2009. Jornal Oficial da União Europeia 204, 15–33.

FAO, 2003. A guide to the seaweed industry. In: McHugh, D.J. (Ed.), FAO Fisheries Technical Paper. No. 441. Rome. 105p.

FAO, 2009. The State of the World Fisheries and Aquaculture. Food and Agricultural Organization of the United Nations, Rome978-92-5-106029-2.

Figueroa, F.L., Bueno, A., Korbee, N., Santos, R., Mata, L., Schuenhoff, A., 2008. Accumulation of Mycosporine-like Amino Acids in *Asparagopsis armata* grown in tanks with fishpond effluents of Gilthead Sea Bream, *Sparus aurata*. Journal of the World Aquaculture Society 39, 692–699.

Figueroa, F.L., Israel, A., Neori, A., Malta, E.J., Martínez, B., Put, A., Inken, S., Marquardt, R., Abdala, R.T., Korbee, N., 2010. Effect of nutrient supply on response of photosynthesis and pigmentation to short-term stress (temperature and UV radiation) in *Gracilaria conferta* (Rhodophyta). Marine Pollution Buletin 60, 1768–1778.

Fleurence, J., 1999. Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science and Technology 10, 25–28.

Friedlander, M., Levy, I., 1995. Cultivation of *Gracilaria* in outdoor tanks and ponds. Journal of Applied Phycology 7, 315–327.

Friedlander, M., 2001. Inorganic nutrition in pond cultivated *Gracilaria conferta* (Rhodophyta): nitrogen, phosphate and sulfate. Journal of Applied Phycology 13, 279–286.

Givernaud, T., El Gourji, A., Mouradi-Givernaud, A., Lemoine, Y., Chiadmi, N., 1999. Seasonal variations of growth and agar composition of *Gracilaria multipartita* harvested along the Atlantic coast of Morocco. Hydrobiologia 399, 167–172.

Haglund, K., Pedersen, M., 1993. Outdoor pond cultivation of the subtropical marine red alga *Gracilaria tenuistipitata* in brackish water in Sweden. Growth, nutrient uptake, co-cultivation with rainbow trout and epiphyte control. Journal of Applied Phycology 5, 271–284.

Halling, C., Aroca, G., Cifuentes, M., Buschmann, A.H., Troell, M., 2005. Comparison of suspended cultivation methods of *Gracilaria chilensis* in an integrated seaweed and fish cage culture. Aquaculture International 13, 409–422.

Hanisak, M.D., 1990. The use of *Gracilaria tikvahiae* (Gracilariales, Rhodophyta) as a model system to understand the nitrogen nutrition of cultured seaweeds. Hydrobiologia 204 (205), 79–87.

Hanisak, D., 1987. Cultivation of *Gracilaria* and other macroalgae in Florida for energy Production. In: Bird, K., Benson, P. (Eds.), Seaweed Cultivation for Renewable Resources. Elsevier, Amsterdam, pp. 191–218.

Hardy, R.W., Tacon, A.G.J., 2002. Fish meal: historical uses, production trends and future outlook for sustainable supplies. In: Stickney, R.R., McVey, J.P. (Eds.), Responsible marine aquaculture. CABI International, pp. 311–325.

Harrison, P.J., Hurd, C.L., 2001. Nutrient physiology of seaweeds: application of concepts to aquaculture. Cahiers de Biologie Marine 42, 71–82.

Hayashi, L., Yokoya, N.S., Ostini, S., Pereira, R.T.L., Braga, E.S., Oliveira, E.C., 2008. Nutrients removed by *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in integrated cultivation with fishes in re-circulating water. Aquaculture 277, 185–191.

He, P.M., Xu, S.N., Zhang, H.Y., Wen, S.S., Dai, Y.J., Lin, S.J., Yarish, C., 2008. Bioremediation efficiency in the removal of dissolved inorganic nutrients by the red seaweed, *Porphyra yezoensis*, cultivated in the open sea. Water Research 42, 1281–1289.

Holmer, M., Hansen, P.K., Karakassis, I., Borg, J.A., Schembri, P.J., 2008. Monitoring of environmental impacts of marine aquaculture. In: Holmer, M., Black, K., Duarte, C.M., Marbà, N., Karakassis, I. (Eds.), Aquaculture in the Ecosystem. Springer, pp. 47–85.

Hurd, C.L., 2000. Water motion, marine macroalgal physiology, and production. Journal of Phycology 36, 453–472.

Israel, A., Martinez-Goss, M., Friedlander, M., 1999. Effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata var. liui* in laboratory and outdoor cultivation. Journal of Applied Phycology 11, 543–549.

Jiang, Z.J., Fang, J.G., Mao, Y.Z., Wang, W., 2010. Eutrophication assessment and bioremediation strategy in a marine fish cage culture area in Nansha Bay, China. Journal of Applied Phycology 22, 421–426.

Jones, A.B., Dennison, W.C., Preston, N.P., 2001. Integrated treatment of shrimp effluent by sedimentation, oyster filtration and macroalgal absorption: a laboratory scale study. Aquaculture 193, 155–178.

Jones, A.B., Preston, N.P., Dennison, W.C., 2002. The efficiency and condition of oysters and macroalgae used as biological filters of shrimp pond effluent. Aquaculture Research 33, 1–19.

Kaushik, S.J., Covès, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391–404.

Kim, J.K., Kraemer, G.P., Neefus, C.D., Chung, I.K., Yarish, C., 2007. Effects of temperature and ammonium on growth, pigment production and nitrogen uptake by four species of *Porphyra* (Bangiales, Rhodophyta) native to the New England coast. Journal of Applied Phycology 19, 431–440.

Lindahl, O., Kolberg, S., 2006. Sweden. In: McVey, J.P., Lee, C.-S., O'Bryen, P.J. (Eds.), Aquaculture and Ecosystems: An Integrated Coastal and Ocean Management Approach. The World Aquaculture Society, Baton Rouge, Louisiana, 70803. United States, pp. 183–189.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S., Svensson, J., Svensson, S., Syversen, U., 2005. Improving marine water quality by mussel farming: a profitable solution for Swedish society. Ambio 34, 131–138.

Lobban, C.S., Harrison, P.J., 1997. Seaweed Ecology and Physiology. Cambridge University Press. 366 pp.

Lüning, K., Pang, S.J., 2003. Mass cultivation of seaweeds: current aspects and approaches. Journal of Applied Phycology 15, 115–119.

Lüning, K., 1990. Seaweeds — their environment, biogeography, and ecophysiology. In: Yarish, C., Kirkman, H. (Eds.), Edited Translation of the German language edition Meeresbotanik: Verbreitung, Okophysiologie und Nutzung der marinen Makroalgen by Klaus Luening. John Wiley and Sons, Inc, New York. 527 pp.

Marinho-Soriano, E., Morales, C., Moreira, W.S.C., 2002. Cultivation of *Gracilaria* (Rhodophyta) in shrimp pond effluents in Brazil. Aquaculture Research 33, 1081–1086.

Marinho-Soriano, E., Camara, M.R., Carbajal, T.M., Carneiro, M.A.A., 2007. Preliminary evaluation of the seaweed *Gracilaria cervicornis* (Rhodophyta) as a partial substitute for the industrial feeds used in shrimp (*Litopenaeus vannamei*) farming. Aquaculture Research 38, 182–187.

Marinho-Soriano, E., Panucci, R.A., Carneiro, M.A.A., Pereira, D.C., 2009. Evaluation of *Gracilaria caudata* J. Agardh for bioremediation of nutrients from shrimp farming wastewater. Bioresource Technology 100, 6192–6198.

Martinez, L.A., Buschmann, A.H., 1996. Agar yield and quality of *Gracilaria chilensis* (Gigartinales, Rhodophyta) in tank culture using fish effluents. Hydrobiologia 327, 341–345.

Martínez-Aragón, J.F., Hernández, I., Pérez-Lloréns, J.L., Vázquez, R., Vergara, J.J., 2002. Biofiltering efficiency in removal of dissolved nutrients by three species of estuarine macroalgae cultivated with sea bass (*Dicentrarchus labrax*) waste waters 1. Phosphate. Journal of Applied Phycology 14, 365–374.

Mata, L., Silva, J., Schuenhoff, A., Santos, R., 2006. The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasporophyte (*Falkenbergia rufolanosa*), cultivated in tanks. Aquaculture 252, 12–19.

Matos, J., Costa, S., Rodrigues, A., Pereira, R., Sousa Pinto, I., 2006. Experimental integrated aquaculture of fish and red seaweeds in Northern Portugal. Aquaculture 252, 31–42.

Mendiguchía, C., Moreno, C., Mánuel-Vez, M.P., García-Vargas, M., 2006. Preliminary investigation on the enrichment of heavy metals in marine sediments originated from intensive aquaculture effluents. Aquaculture 254, 317–325.

Mente, E., Pierce, P.J., Santos, M.B., Neofitou, C., 2006. Effect of feed and feeding in the culture of salmonids on the marine aquatic environment: a synthesis for European aquaculture. Aquaculture International 14, 499–522.

Nagler, P.L., Glenn, E.P., Nelson, S.G., Napolean, S., 2003. Effects of fertilization treatment and stocking density on the growth and production of the economic seaweed *Gracilaria parvispora* (Rhodophyta) in cage culture at Molokai, Hawaii. Aquaculture 219, 379–391.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature 405, 1017–1024.

Nelson, G.S., Glenn, E.P., Conn, J., Moore, D., Walsh, T., Akutagawa, M., 2001. Cultivation of *Gracilaria parvispora* (Rhodophyta) in shrimp-farm effluent ditches and floating cages in Hawaii: a two-phase polyculture system. Aquaculture 192, 239–248.

Neori, A., Ragg, N.L.C., Shpigel, M., 1998. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: II. Performance and nitrogen partitioning within an abalone (*Haliotis tuberculata*) and macroalgae culture system. Aquaculture Engineering 17, 215–239.

Neori, A., Shpigel, M., Ben-Ezra, D., 2000. Sustainable integrated system for culture of fish, seaweed and abalone. Aquaculture 186, 279–291.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture 231, 361–391.

Neori, A., Troell, M., Chopin, T., Yarish, C., Critchley, A., Buschmann, A., 2007. The need for a balanced ecosystem approach to Blue Revolution Aquaculture. Environment 49, 38–42.

Nobre, A.M., Robertson-Andersson, D., Neori, A., Sankar, K., 2010. Ecological economic assessment of aquaculture options: comparison between abalone monoculture and integrated multi-trophic aquaculture of abalone and seaweeds. Aquaculture 306, 116–126.

Nyberg, C.D., Wallentinus, I., 2009. Long-term survival of an introduced red alga in adverse conditions. Marine Biology Research 5, 304–308.

Nyberg, C.D., Thomsen, M.S., Wallentinus, I., 2009. Flora and fauna associated with the introduced red alga *Gracilaria vermiculophylla*. European Journal of Phycology 44, 395–403.

Oliveira, E.C., Alveal, K., Anderson, R.J., 2000. Mariculture of the agar-producing Gracilarioid red algae. Reviews in Fisheries Science 8, 345–377.

Peng, C., Hong-BO, S., Di, X., Song, Q., 2009. Progress in *Gracilaria* biology and developmental utilization: main issues and prospective. Reviews in Fisheries Science 17, 494–504.

Pereira, R., Kraemer, G., Yarish, C., Sousa-Pinto, I., 2008. Nitrogen uptake by gametophytes of *Porphyra dioica* (Bangiales, Rhodophyta) under controlled-culture conditions. European Journal of Phycology 43, 107–118.

Pereira, R., Abreu, M.H., Valente, L., Rema, P., Sousa Pinto, I., 2010. Production of seaweeds in integrated multi-trophic aquaculture for application as ingredients in fish meal. XXth International Seaweed Symposium book of abstracts, p. 86. Ensenada, Mexico.

Piedrahita, R.H., 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226, 35–44.

Ramseyer, L., Garling, D., 1997. Fish nutrition and aquaculture waste management. Illinois–Indiana Sea Grant Program, Publication CES-305.

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., Boyne-Travis, S., 2007. Integrated Multi-Trophic Aquaculture (IMTA): a potential strategic choice for farmers. Aquaculture Economics & Management 11, 99–110.

Rueness, J., 2005. Life histories and molecular sequences of *Gracilaria vermiculophylla*. Phycologia 44, 120–128.

Ryther, J.H., Goldman, J.C., Gifford, J.E., Huguenin, J.E., Wing, A.S., Clarner, J.P., Williams, L.D., Lapointe, B.E., 1975. Physical models of integrated waste recycling-marine polyculture systems. Aquaculture 5, 163–177.

Sanderson, J.C., Cromey, C.J., Dring, M.J., Kelly, M.S., 2008. Distribution of nutrients for seaweed cultivation around salmon cages at farm sites in north–west Scotland. Aquaculture 278, 60–68.

Saunders, G.W., 2009. Routine DNA barcoding of Canadian Gracilariales (Rhodophyta) reveals the invasive species *Gracilaria vermiculophylla* in British Columbia. Molecular Ecology Resources 9, 140–150.

Shpiggel, M., Neori, A., 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. Aquacultural Engineering 15, 313–326.

Silva, J.F., 1985. O moliço da Ria de Aveiro. Jornadas da Ria de Aveiro, II. Câmara Municipal da Ria de Aveiro, Portugal, pp. 223–251.

Silva, J.F., Duck, R.W., Hopkins, T.S., Rodrigues, M., 2002. Evaluation of the nutrient inputs to a coastal lagoon: the case of the Ria de Aveiro, Portugal. Hydrobiologia 475 (476), 379–385.

Silva, J.F., Duck, R.W., Catarino, J.B., 2004. Seagrasses and sediment response to changing physical forcing in a coastal lagoon. Hydrology and Earth System Sciences 8, 151–159.

Silva, J.M.G., Espe, M., Conceição, L.E.C., Dias, J., Valente, L.M.P., 2009. Senegalese sole juveniles (*Solea senegalensis* Kaup, 1858) grow equally well on diets devoid of fish meal provided the dietary amino acids are balanced. Aquaculture 296, 309–317.

Smit, A.J., 2002. Nitrogen uptake by *Gracilaria gracilis* (Rhodophyta): adaptations to a temporally variable nitrogen environment. Botanica Marina 45, 196–209.

Soler-Vila, A., Coughlan, S., Guiry, M.D., Kraan, S., 2009. The red alga *Porphyra dioica* as a fish-feed ingredient for rainbow trout (*Oncorhynchus mykiss*): effects on growth, feed efficiency, and carcass composition. Journal of Applied Phycology 21, 617–624.

Sousa, A.M.M., Alves, V.D., Morais, S., Delerue-Matos, C., Gonçalves, M.P., 2010. Agar extraction from integrated multitrophic aquacultured *Gracilaria vermiculophylla*: evaluation of a microwave-assisted process using response surface methodology. Bioresource Technology 101, 3258–3267.

Troell, M., Halling, C., Nilsson, A., Buschmann, A.H., Kautsky, N., Kautsky, L., 1997. Integrated open sea cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta) and salmons for reduced environmental impact and increased economic output. Aquaculture 156, 45–62.

Troell, M., Halling, C., Neori, A., Buschmann, A.H., Chopin, T., Yarish, C., Kautsky, N., 2003. Integrated mariculture: asking the right questions. Aquaculture 226, 69–80.

Valente, L.M.P., Gouveia, A., Rema, P., Matos, J., Gomes, E.F., Pinto, I.S., 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. Aquaculture 25, 85–91.

Villanueva, M.D., Sousa, A.M.M., Gonçalves, M.P., Nilsson, M., Hiliiou, L., 2009. Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). Journal of Applied Phycology 22, 211–220.

Wik, T.E.I., Linden, B.T., Wramner, P.I., 2009. Integrated dynamic aquaculture and wastewater treatment modelling for recirculating aquaculture systems. Aquaculture 287, 361–370.

Yang, Y.F., Fei, X.G., Song, J.M., Hu, H.Y., Wang, J.C., Chung, I.K., 2006. Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. Aquaculture 254, 248–255.

Yarish, C., Pereira, R., 2008. Mass production of Marine Macroalgae. In: Sven Erik Jørgensen, S.E., Fath, B.D. (Eds.), Ecological Engineering. : Encyclopedia of Ecology, 5 vols. Elsevier, Oxford, pp. 2236–2247.

Yokoya, N.S., Kakita, H., Obika, H., Kitamura, T., 1999. Effects of environmental factors and plant growth regulators on growth of the red alga *Gracilaria vermiculophylla* from Shikoku Island, Japan. Hydrobiologia 398 (399), 339–347.

Zertuche-González, J.A., Camacho-Ibar, V.F., Pacheco-Ruiz, I., Cabello-Pasini, A., Galindo-Bect, L.A., Guzman-Calderon, J.M., Macias-Carranza, V., Espinoza-Avalos, J., 2009. The role of *Ulva* spp. as a temporary nutrient sink in a coastal lagoon with oyster cultivation and upwelling influence. Journal of Applied Phycology 21, 729–736.

Zou, D., Xia, J., Yang, Y., 2004. Photosynthetic use of exogenous inorganic carbon in the agarophyte *Gracilaria lemaneiformis* (Rhodophyta). Aquaculture 237, 421–431.

Zou, D.H., Gao, K.S., 2009. Effects of elevated CO2 on the red seaweed *Gracilari lemaneiformis* (Gigartinales, Rhodophyta) grown at different irradiance levels. Phycologia 48, 510–517.

Web references

http://seagrant.gso.uri.edu/sustainable_seafood/ecolabeling.html.

www.oilalgae.com.