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Traditional vs. Integrated Multi-Trophic Aquaculture of *Gracilaria chilensis* C. J. Bird, J. McLachlan & E. C. Oliveira: Productivity and physiological performance

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ABSTRACT

Integrated Multi-Trophic Aquaculture (IMTA) systems are designed to mitigate the environmental problems caused by several forms of fed aquaculture. *Gracilaria chilensis* is commercially cultivated in Chile and experimental studies recommend it as an efficient biofilter in IMTA systems. Traditional bottom culture *Gracilaria* farms face production problems mainly related to the cultivation system and seasonal changes in nitrogen availability and irradiance. IMTA may offer a solution to some of these problems. This study intended to investigate the productivity of *G. chilensis* near salmon farms and assess its nitrogen removal and photosynthetic performance. The most appropriate cultivation methodologies (i.e. floating long-lines vs. bottom cultivation) for *Gracilaria* production were also evaluated. During austral summer and autumn, 3 long-line cultivation units were set at different distances from a salmon farm, one of them being away from the influence of salmonid aquaculture. Additionally, a similar cultivation unit was installed as a traditional bottom culture.

Gracilaria growth performance was always higher on the suspended cultures near the salmon cages. Summer daily mean growth rates at those sites reached 4% (±0.29) with a mean biomass production of over 1600 g m^{-2} $month^{-1}$(±290) which was double the unimpacted site. The productivity of bottom cultured *Gracilaria* was highly reduced by biomass losses. N removal and photosynthetic performances provided possible explanations for the differences found. The long-line cultivation unit proved to be the most efficient technology for nutrient removal with monthly removal of up to 9.3 g (±1.6) N per meter of long-line. The proximity to the salmon farm also mitigated the decrease in photosynthetic activity after the midday irradiance peak. *G. chilensis* at those sites maintained daily average values of Φ_{PSII} around 0.6 and rETR close to 40 µmol e^{-} m^{-2} s^{-1}. *Fv/Fm* values (0.6) were similar at all cultivation areas. Our results clearly indicated the advantages of integrating *G. chilensis* aquaculture with salmon farms. Within the IMTA system, the productivity and physiological performance of *G. chilensis* were greatly improved and this seaweed's biofiltration efficacy was confirmed. We suggest that a 100 ha *G. chilensis* long-line system will effectively (ca. 100%) reduce the N inputs of a 1500 tonnes salmon farm.

1. Introduction

During the last 10 to 15 years, claims for the use of ecological engineering tools towards the development of a more sustainable aquaculture have increased (Chopin et al., 2008; Crab et al., 2007; Naylor et al., 2000). Up to 60% of the nitrogen from salmonid fish feeds may remain in the environment (McVey et al., 2002; Mente et al., 2006; Sanderson et al., 2008). In this context, seaweed based Integrated Multi-Trophic Aquaculture (IMTA) systems has been proposed (Buschmann et al., 2008a,c; Chopin et al., 2001, 2008; Neori et al., 2004, 2007; Troell et al., 2003). In this farming approach, seaweeds and other extractive organisms (e.g. filter-feeding invertebrates) convert dissolved inorganic and suspended organic nutrients, produced by fed aquaculture (e.g. finfish and shrimp), into additional crops. However, to encourage stakeholder investment (finfish and seaweed farmers) it will be necessary to design profitable and environmentally sustainable systems which couple nutrient removal efficiency with high productivity (Buschmann et al., 2001a,b, 2008c; Tacon and Halwart, 2007; Troell et al., 2003). To achieve these goals it will be important to recognize the primary economic driver of the IMTA system (i.e. the fed component) and the opportunities of using extractive components that are inherently valuable commodities (e.g. *Porphyra*, *Gracilaria*, *Kappaphycus*, *Macrocystis* (Blouin

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et al., 2007; Buschmann et al., 2008c; Hayashi et al., 2008; Matos et al., 2006; Pereira et al., 2006; Rodrigueza and Montaño, 2007; Troell et al., 2006).

Gracilaria is one of the seaweed genera most exploited worldwide (Yarish and Pereira, 2008). Cultivation of the agarophyte, *Gracilaria chilensis* Bird, McLachlan & Oliveira, has been a commercial activity since mid 80s along the Chilean coast (Buschmann et al., 2001b). This species is cultivated in shallow bays, estuaries as well as on intertidal mudflats propagated by thalli fragmentation. Nonetheless, this traditional bottom cultivation method carries inherent disadvantages, mainly related to biomass losses and variability in seasonal production (Buschmann et al., 1995, 2001b; Santelices and Ugarte, 1987). A limited number of experimental studies have demonstrated the enhanced productivity of *G. chilensis* when cultivated in long-lines near salmon farms (Buschmann et al., 2008c; Halling et al., 2005; Troell et al., 1997).

Photosynthesis may be used as an estimate of environmental effects on seaweeds productivity (Davison, 1991; Kraemer and Yarish, 1999; Kraemer et al., 2002). In addition to the effects of light and temperature, nitrogen availability may impact photosynthesis (Cabello-Pasini and Figueroa, 2005; Henley et al., 1991; Huovinen et al., 2006; Longstaff et al., 2002; Parkhill et al., 2001). Chlorophyll fluorescence may then also be a valuable monitoring tool to assess the physiological performance of seaweed in aquaculture systems (Cabello-Pasini et al., 2000). The use of Pulse Amplitude Modulated (PAM) fluorometry is now widely accepted for it being an efficient and rapid assessment of *in situ* physiological performance of macrophytes (Beer et al., 2000; Gómez et al., 2004; Lamote et al., 2007). Episodic events that lead to nitrogen stress in outdoor seaweed cultures have been monitored by looking at the maximum efficiency of PSII (Fv/Fm) (Buschmann et al., 2008c; Figueroa et al., 2006; Mata et al., 2006). However, to relate Fv/Fm to the seaweed growth rates and nitrogen removal capacity in IMTA systems is still uncertain.

The objectives of this study were 1) to investigate the nitrogen removal capacity and the photosynthetic performance of *G. chilensis* in an IMTA system, 2) to establish the most effective form of *Gracilaria*

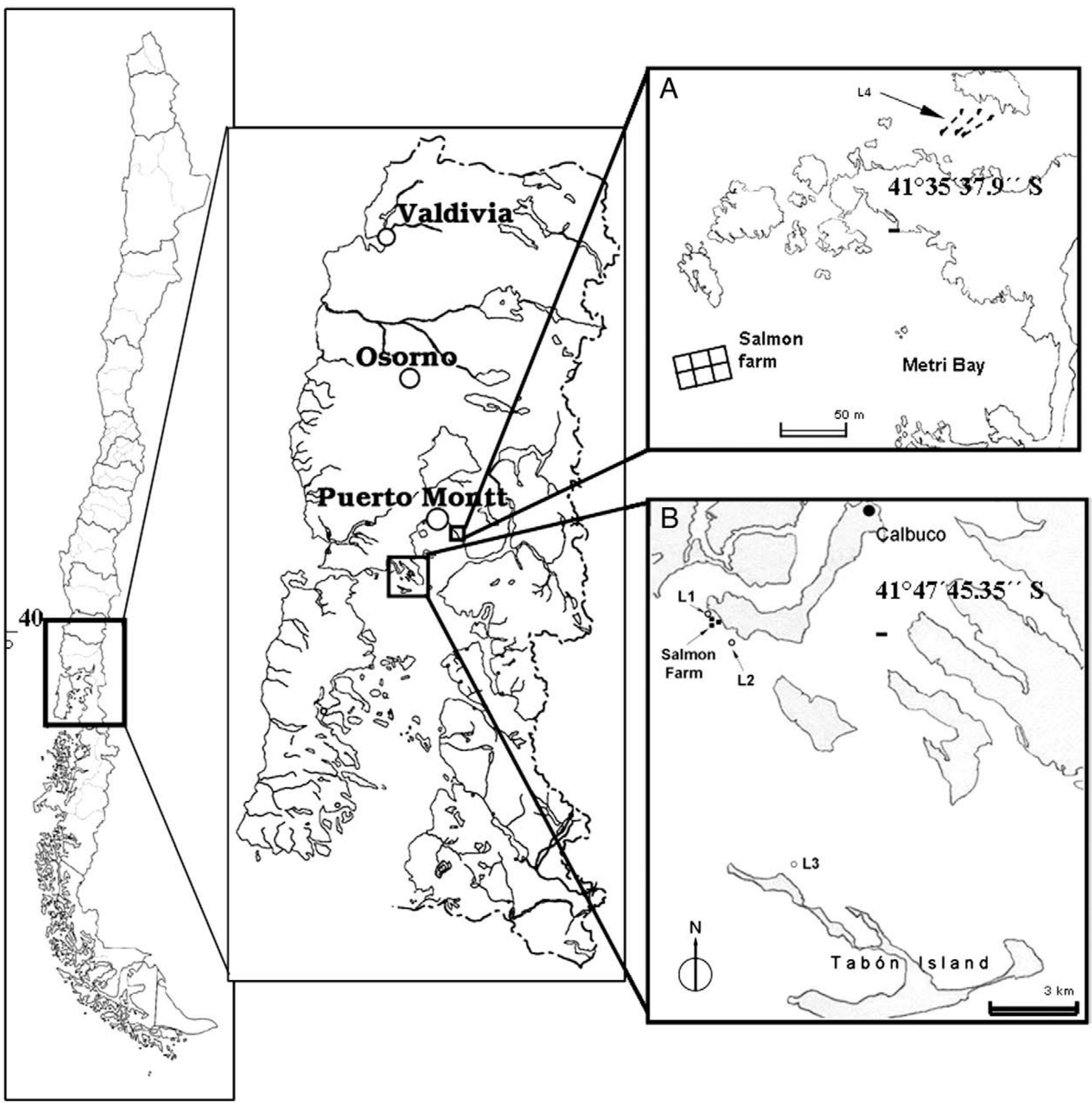

Fig. 1. Map showing the cultivation sites in southern Chile. A. Bottom cultivation unit set at Metri Bay (L4). B Cultivation units set on long-lines systems at 100 m (L1), 800 m (L2) and 7 km (L3) from Trusal's salmon farm at Calbuco.

cultivation in the vicinity of salmon farms. In addition, we intended to collect data that could provide useful information for finfish and seaweed farmers, together with environmental policy makers.

2. Methodology

2.1. Localization and features of the experimental cultivation units

The experimental culture of *G. chilensis* was carried out during 2007, at Los Lagos region, in southern Chile (Fig. 1). Three long-line cultivation units were set within different distances from a salmon farm. At Calbuco (41° 48′ S; 73° 11′ W) the closest units (L1 — 100 m and L2 — 800 m) were set in order to receive the main flow of nutrient discharges from salmon cage systems during the flood tides (average currents: 7.6 and 2.4 cm s^{-1}, during the flood and ebb periods, respectively). Next to Isla Tabón (41° 52′ S; 73° 09′ W), a non-impacted salmon culture area traditionally used by *Gracilaria* farmers, a third cultivation unit (L3) was placed 7 km away from the salmon farm (Fig. 1A).

During the austral summer (January through March), a fourth long-line ("seeded" rope) experimental cultivation unit (L4) was set in a mud-flat at Metri (41° 36′ S 72° 42′ W) (Fig. 1B). This was done in order to compare the suspended culture of *G. chilensis* with the traditional bottom culture (Buschmann et al., 1995; Sahoo and Yarish, 2005). Due to administrative regulations and logistics it was impossible to establish replicate long-lines in all treatments; we tried to minimize this lack of large scale replication by dividing the 100 m long-lines in independent sections and haphazardly assign them 3 replicate rope sections.

The cultivation depth was about 1 m for the suspended culture units (Fig. 2), as described by Buschmann et al. (2008c). The bottom culture system was more exposed to the influence of tidal cycles.

2.2. Characterization of the cultivation sites

Environmental parameters were monitored at all cultivation sites (see Table 1). Photosynthetic Active Radiation (PAR) levels were monitored with a photometer Li-Cor 182 and water temperatures were measured with a diving PAM (Diving-PAM, Walz-Germany). Water samples were collected from 1 m depth at each cultivation site and filtered at the lab prior to analysis. Ammonia analysis was initiated at the day of collection by the phenol method (Solorzano, 1969). Samples were kept refrigerated until the next day and then analysed for nitrate by the cadmium column method (Strickland and Parsons, 1972).

Table 1
Environmental parameters measured at each cultivation site.

	L1	L2	L3	L4
MRT (°C)	14.7–13.7	14.7–13.7	15–12.8	15.4–15.5
MSST (°C)	16.1–13.9			
Incident irradiance (μmol m^{-2} s^{-1})	1522.3–1231.33			
NO_3^- (μM)	1.80–23.87	2.18–20.79	1.85–23.85	1.071 (summer)
NH_4^+ (μM)–summer	1.68	1.29	3.60	3.39

Mean values for summer–autumn. MRT — Mean registered temperature at each sampling moment; MSST — Mean Sea Surface Temperature at Puerto Montt (41°29′ S72°57′W), data from www.shoa.cl (Servicio Hidrografico y Oceanografico de la Armada de Chile).

2.3. Growth assessment

In early January 2007 (austral summer), at each cultivation unit, three rope sections (3 m long each) were haphazardly distributed and seeded every 0.2 m with 50 g *Gracilaria* bundles ($n = 45$) (Fig. 2). The stocking biomass was collected from the same natural population at Metri Bay (Fig. 1). After 2 months of culture, as suggested by Halling et al. (2005), all *Gracilaria* bundles remaining in the 3 m rope sections were drained to remove the excess superficial water, weighted (±1 g) and reduced to 100 g. The relative growth rate was determined by the equation:

$$RGR(\%) = \ln(FW) - \ln(IW) / T*100$$

where FW = Final fresh weight, IW = Initial fresh weight and T = Days in culture. The productivity for each cultivation unit was determined by the monthly increase in biomass (g) per each meter of rope. This procedure was also repeated in the austral autumn (March–April).

2.4. Measurement of photosynthetic parameters

During summer (January–March), photosynthetic measurements were taken at the field and laboratory, using underwater pulse modulation fluorometry (Diving-PAM, Walz). After several trials made during the experiment, one date and photon fluence level were selected to do the comparative analysis between the cultivation sites.

The daily behaviour of the effective quantum yield of PSII (Φ_{PSII}) and of the relative electron transport rate (rETR) was assessed for 30 *Gracilaria* bundles haphazardly chosen from the 3 experimental sections. Measurements of the maximal quantum yield of PSII (Fv/Fm), were made before and after the daily irradiance peak, on dark adapted (30 min) *Gracilaria* branches ($n = 30$) grown at sites L1, L2 and L3.

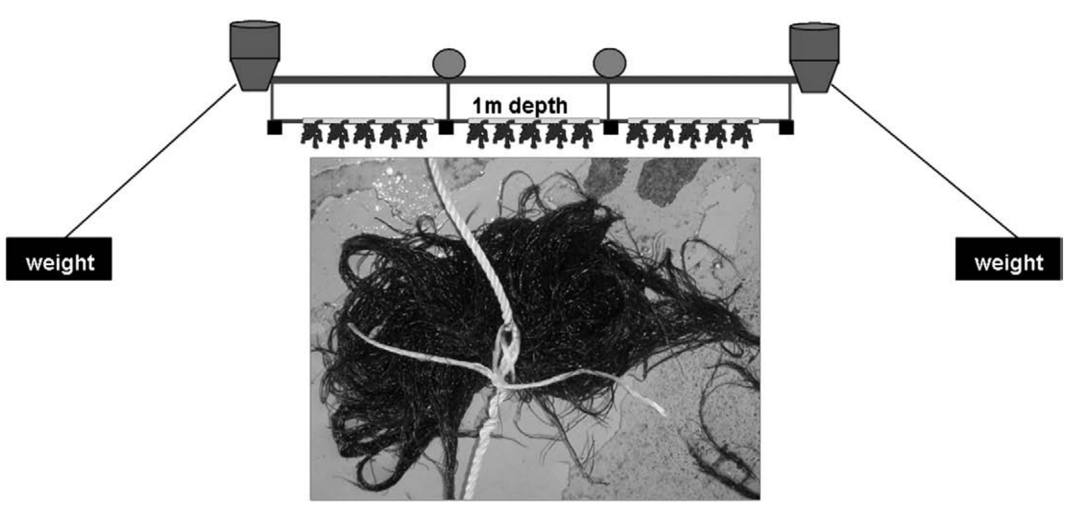

Fig. 2. Scheme of the experimental suspended cultivation units and detail of a *Gracilaria chilensis* bundle held within the cultivation rope.

Additionally, *Gracilaria* samples were collected ($n=10$), transported to the laboratory in a cooler box and kept in similar water temperature (14 °C) and under dim light conditions. Less than 24 h after collection, photosynthesis vs. light curves (*P–I*) were obtained through Rapid Light Curves (RLC) generated with increasing light intensities up to 430 μmol photons m^{-2} s^{-1}. During this process, the seaweed material was kept in a thermal bath around 14 °C. The effective quantum yield of PSII (Φ_{PSII}) was obtained for each given light pulse and the electron transport rate (ETR) was calculated as:

$$ETR = \Phi_{PSII} * I_{PAR} * A * 0.5 \qquad \text{(Genty et al., 1989)}$$

where I_{PAR} is the incident irradiance, A is the absorbance factor measured for each replica and 0.5 is the assumed proportion of photons absorbed by pigments associated with PSII.

The estimation of the photosynthetic parameters was attained by fitting the data set to the non-linear function modified after Jassby and Platt (1976):

$$ETR = ETR_{max} * \tanh(\alpha * I_{PAR} / ETR_{max})$$

where ETR_{max} is the maximal ETR, tanh is the hyperbolic tangent function, α is the initial slope of the *P–I* curve which translates the electron transport efficiency at low irradiances and I_{PAR} the incident irradiance. E_k, defining the saturation irradiance for electron transport was calculated as the interception between α and ETR_{max}.

2.5. Nitrogen tissue analysis

G. chilensis tissue samples were collected for N analysis at the beginning and end of each cultivation period. For preservation, they were lyophilized and kept in the cold. At the Seaweed Marine Biotechnology Lab (UConn), the samples were kept in the oven (40 °C) for 24 h to eliminate any residual moisture and analysed for total N tissue content (% dry weight) using a CHNS/O Analyser (Series II, 2400 Perkin-Elmer).

2.6. Statistical analysis

A two-way ANOVA was used to analyse the influence of the cultivation site and time of measurement on the daily cycles of Φ_{PSII} and rETR. *A posteriori* differences were evaluated with the Unequal N HSD test (Statistica 6.0, StatSoft). All other effects were analysed with a one-way ANOVA. Data were tested for variance heterogeneity (Cochran's Test) and, when the answer was significant, the data were transformed (Log or ArcSin). When the transformation didn't normalize the data, having a high number of replicates, we used a more conservative level of significance $P<0.01$ (Underwood, 1997). The differences found were analysed *a posteriori* with a Student–Newman Keuls test.

3. Results

3.1. Growth and productivity

During the entire course of the experiment, the general growth performance of *G. chilensis* cultivated on suspended long-lines, near the salmon cages, was always better than at the control site Isla Tabón (L3) and at the bottom culture site at Metri (L4) (Fig. 3). *G. chilensis* presented higher growth rates during the summer, coincident with higher water temperatures and photon fluence rates measured at that

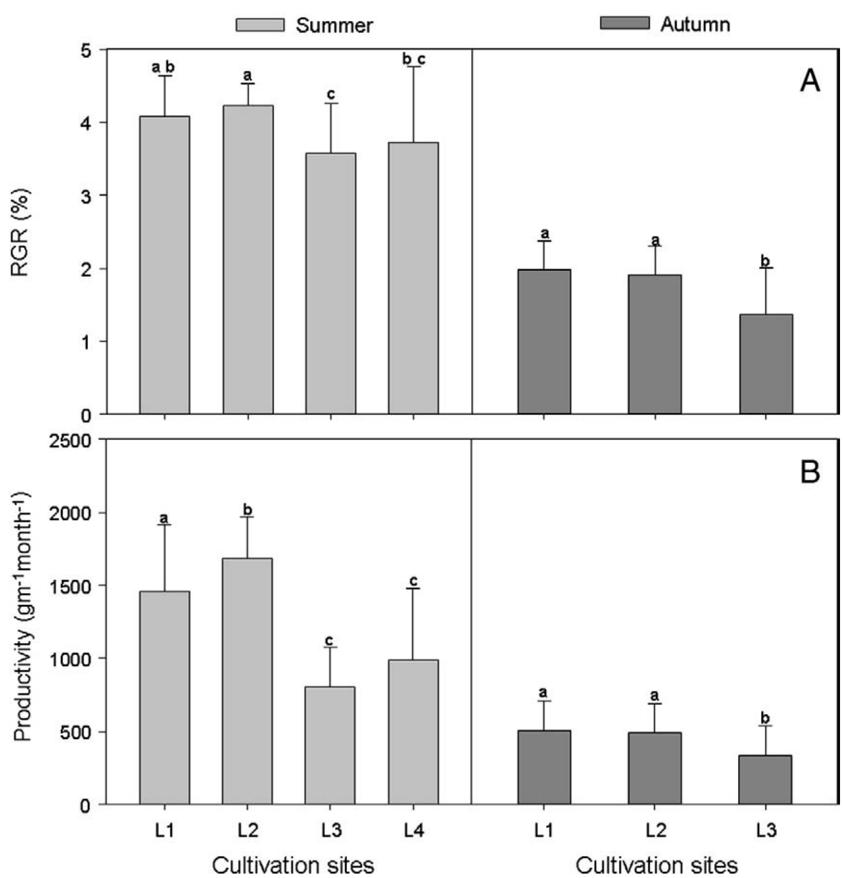

Fig. 3. Summer and autumn growth patterns (mean ± (SE), $n=30$) of *Gracilaria chilensis* cultivated at different distances from the salmon cages (L1 = 100 m, L2 = 800 m, L3 = 7000 m and L4 = bottom culture). A. Relative Growth Rate (% day^{-1}) and B. Productivity (g m^{-1} $month^{-1}$). Letters indicate the groups differentiated by posthoc tests, when these were significant at $P<0.01$.

Fig. 4. Detail of *Gracilaria chilensis* plants after 2 months in culture at L2.

time (Table 1). Both, in summer ($F_{(3,\ 116)}=5.62$, $P<0.01$) and autumn ($F_{(2,\ 87)}=11.87$, $P<0.01$), *G. chilensis* plants cultivated close to the salmon farm (L1 and L2) showed significantly the highest growth rates with a daily weight increase above 4% (summer) and 2% (autumn). The lowest RGR was always obtained at the cultivation unit (L3) placed 7 km away from the salmon farm, while at the bottom culture area (L4) the growth rates, although not being the lowest (ca. 3.5%), showed the highest variation (Fig. 3A).

G. chilensis productivity values were coincident with RGR results, with summer monthly biomass production (fresh weight) reaching values over 1680 g m^{-1}, at sites near (L2) the salmon cages (Fig. 4). These values were significantly ($F_{(3,116)}=32.47$, $P<0.05$) higher than those obtained either at L3 or L4. The highest productivity at L2 was always coupled with an almost complete absence of epiphytes. In autumn and after the harvest, *G. chilensis* production dropped to 40%, with the closest sites to the salmon farm (L2>L1) still maintaining the highest productivity levels ($F_{(2,\ 87)}=6.59$, $P<0.05$; Fig. 3B).

3.2. Nitrogen removal capacity

Nitrogen water concentrations at the cultivation sites were similar for nitrate sources but quite different in terms of ammonia, with sites away from the salmon cages surprisingly presenting the highest values (Table 1). The analysis for N tissue content of the plants collected in March, showed significant differences between them ($F_{(3,16)}=8.872$, $P<0.05$, Fig. 5A). Contrary to the water analysis results, *G. chilensis* grown at L2 had the highest N content, 3.68% DW, ca. 20% higher than the values observed at Isla Tábon (L3) and Metri (L4). Taking into account the N tissue content of the biomass used to seed all lines (Fig. 5A, white bars), the difference in N tissue storage is significant between the cultivation sites. By combining the nitrogen tissue content with the productivity, it was possible to determine the summer N removal potential for each culture system. The cultivation site had a significant effect for the N removal capacity ($F_{(3,116)}=58.36$, $P<0.05$; Fig. 5B). The site furthest from the salmon cages (L3) together with the bottom culture (L4) showed the lowest N removal capacity. The cultivation unit placed 800 m from the salmon farm (L2) had the highest nitrogen removal capacity each month, ca. 9.3 g N m^{-1} of long-line seeded with *G. chilensis*.

3.3. Photosynthetic performance

The different photosynthetic parameters measured in *G. chilensis* responded differentially to the cultivation treatments. *Fv*/*Fm* of *G. chilensis* did not differ between the cultivation sites ($F_{(2,\ 87)}=0.23$, $P=0.7975$), reaching mean values around 0.6 for all treatments. This value was constant throughout the day, with no significant differences found, in neither the sites (L1: $F_{(1,49)}=9.845$ and L3: $F_{(1,58)}=1.1257$, $P>0.05$), nor between measurements done before or after each day irradiance peak (data not shown).

The daily cycles of Φ_{PSII} and rETR obtained at the different cultivation sites are shown in Fig. 6. In this study's circumstances it was impossible to take photosynthesis measures in the same day for all sites; the visible effect of this is shown in Fig. 6 with the distinct irradiance patterns observed for each sampling day. Despite the high mean irradiance intensity observed (ca. 1300 µmol m^{-2} s^{-1}), our underwater

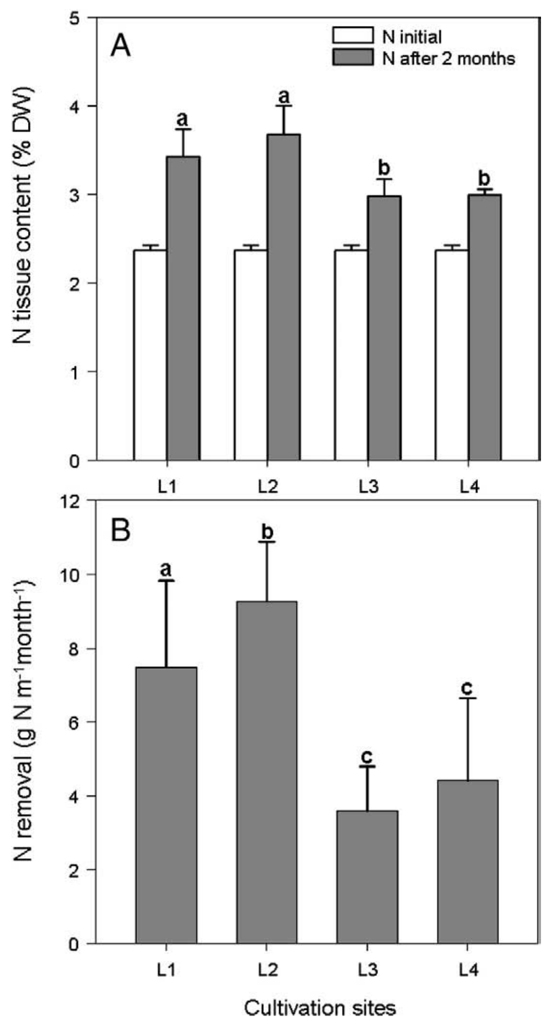

Fig. 5. A. Nitrogen tissue content (% dry weight) of *Gracilaria chilensis*. Mean ± SE, $n=30$. White bar stands for initial N content ($n=6$). Grey bars are after 2 months (summer) in culture at different distances from the salmon cages (L1 = 100 m, L2 = 800 m, L3 = 7000 m and L4 = (bottom culture). B. Nitrogen removal potential (g/m/month) for each cultivation site. Mean ± SE, $n=30$. Letters indicate the groups differentiated by posthoc tests, when these were significant at $P<0.05$.

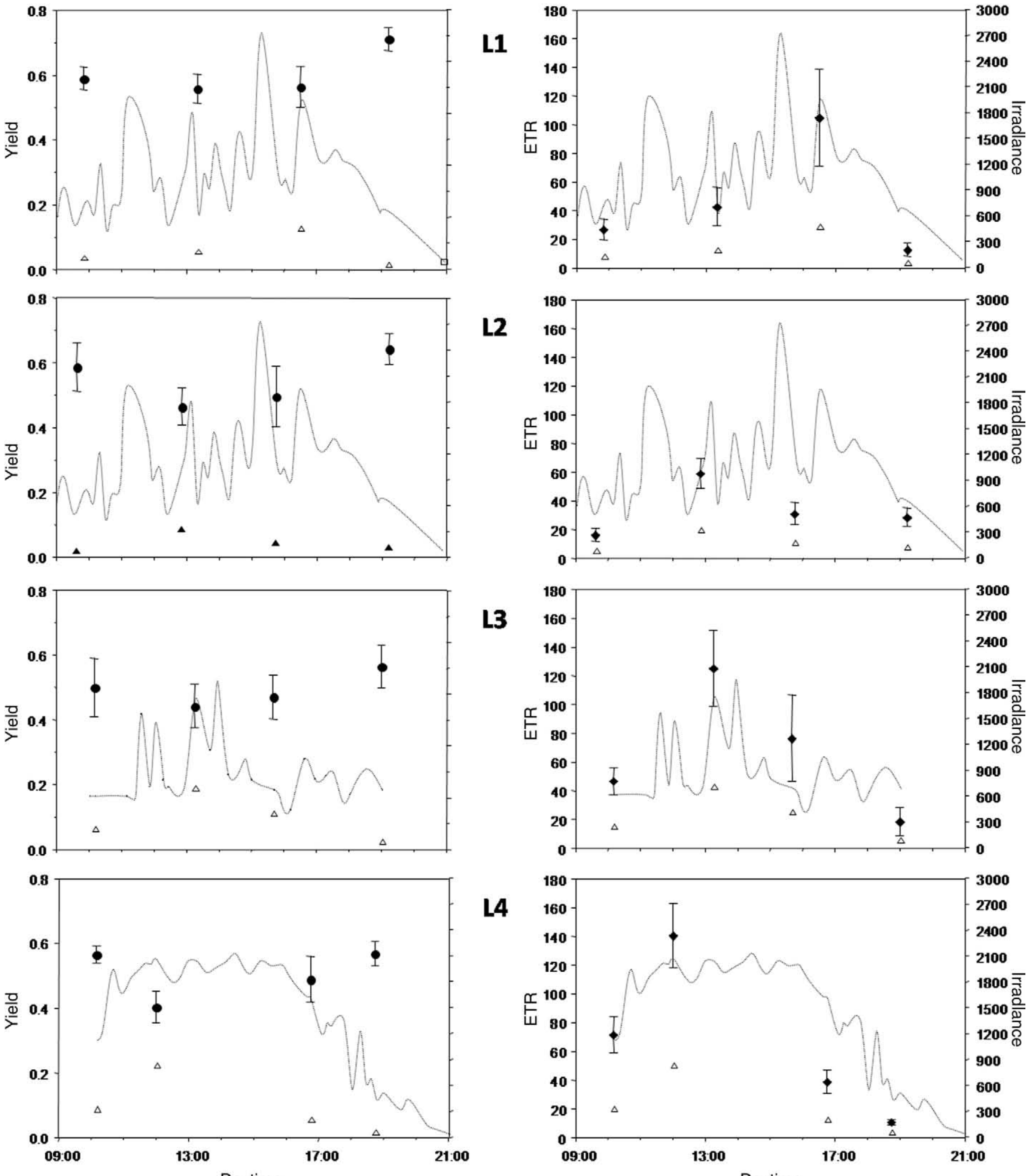

Fig. 6. Summer daily pattern of Yield (Φ_{PSII}; ●) and rETR (♦) for *Gracilaria chilensis* cultivated at the different experimental sites: L1, L2, L3, L4. Mean ± SE, $n = 16–30$. Line stands for daily irradiance measures and open triangles for average PAR measured at 1 m depth.

measurements indicate that only ca. 25% of the incident irradiance (Fig. 6) reaches the cultivation depth and is therefore available for the seaweeds' photosynthetic processes. The cultivation site and daytime at which the measurement was made had a significant influence in the photosynthetic daily behaviour of *G. chilensis*, with the interaction between those factors being also significant (Table 2). *G. chilensis* presented the lowest values of Φ_{PSII} (around 0.44) at sites L3 and L4 with correspondent highest rETR values (140 µmol e^{-} m^{-2} s^{-1}) after the irradiance peak. The tests made *a posteriori* revealed that the photosynthetic responses at these two sites were very identical.

Table 2
Two way-ANOVA results from the analysis of the cultivation site and the daytime on the daily cycles of yield (Φ_{PSII}) and rETR.

Factor	Ss		Df	MS		F	
	Yield	ETR	Yield/ETR	Yield	ETR	Yield	ETR
Site	5.27E+05	2.30E+05	3	1.76E+05	7.67E+04	47.9*	77.3*
Time	9.13E+05	1.32E+06	3	3.04E+05	4.40E+05	83*	443.6*
Time^Site	1.01E+05	6.38E+05	9	1.12E+04	7.09E+04	3.1*	71.5*

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

G. chilensis from the suspended cultivation units close to the salmon farms (L1 and L2), even having a slight drop/rise on Φ_{PSII}/rETR values at midday, maintained daily average values of Φ_{PSII} around 0.6 and rETR close to 40 µmol e^{-} m^{-2} s^{-1}.

Through the Rapid Light Curves (RLC) generated at the laboratory (Fig. 7) we determined ETR_{max}, E_k and α values for *G. chilensis* collected at each cultivation site (Table 3). The α of *G. chilensis* plants collected from L1 reached a mean value of 12%, against values of 8 to 9% in the other sites ($F_{(2,27)}$ = 7.426, P<0.05). The maximal photosynthesis, estimated from ETR_{max}, was significant higher at L1 and L4 ($F_{(2,27)}$ = 15.760, P<0.01). The E_k values were higher (99.65 µmol m^{-2} s^{-1}) for *G. chilensis* plants collected at L4 ($F_{(2,27)}$ = 11.292, P<0.01) than for the other sites.

Table 3
Photosynthetic responses (mean ± (SE), n = 10) of *Gracilaria chilensis* cultivated in long-lines close to a commercial salmon farm (L1), away from it (L3) and by bottom culture (L4).

	L1	L3	L4
α (µmol e^{-1} m^{-2} s^{-1}) (µmol m^{-2} s^{-1})$^{-1}$	0.12 ± 0.02 [a]	0.09 ± 0.019 [b]	0.08 ± 0.02 [b]
ETR_{max} (µmol e m^{-2} s^{-1})	7.23 ± 0.96 [a]	4.67 ± 1.50 [b]	7.78 ± 1.44 [a]
E_k (µmol m^{-2} s^{-1})	63.39 ± 17.11 [a]	53.73 ± 23.42 [a]	99.65 ± 26.76 [b]
Mean irradiance at the day of collection (µmol m^{-2} s^{-1})	1309.56	1309.56	1427.54

Letters indicate the groups differentiated by posthoc tests, when these were significant at P<0.01.

4. Discussion

Aquaculture is an essential activity in the economy of southern Chile with salmon farming being the most important commodity (Buschmann et al., 2006, 2008b; Flores-Aguilar et al., 2007; López, 2008). Salmon aquaculture continues to increase although with serious environmental and economic issues that might affect this industry's sustainability (Barton, 1997; WWF, 2007). IMTA may be part of the solution. Several studies have reported clear advantages of growing seaweeds near fish farms (e.g. Anderson et al., 1999; Buschmann et al., 2008c; Chopin et al., 2008; McVey et al., 2002; Nagler et al., 2003; Troell et al., 1997). Different algal species show distinctive N uptake preferences (Bracken and Stachowicz, 2006; Chopin et al., 2001; Neori et al., 2004). *Gracilaria* species are efficient biofilters due to their ability to efficiently remove both ammonia and nitrate from the culture media (Buschmann et al., 1994, 1996; D'Elia and DeBoer, 1978; Jones et al., 2001; Matos et al., 2006; Neori et al., 2000).

The decision to perform this IMTA study with *G. chilensis* was based on the good results (see Section 1) obtained in previous studies with this species (Buschmann et al., 1996, 2008c; Halling et al., 2005; Troell et al., 1997). We were able to determine that *G. chilensis* maintains high growth performance (average 1.7 kg m^{-2} fresh weight) up to 1 km from a 1 ha salmon farm with a production capacity of 1500 tonnes.

The primary objective of our study was to establish the nitrogen removal capacity of *G. chilensis* in an open water IMTA system. Finfish aquaculture may provide dissolved inorganic N to the seaweeds during periods of N limitation and high PAR (austral summer). The water samples from each cultivation site revealed the highest ammonia concentrations at sites away from the salmon cages, what was not expected. One must account for the fact that these were isolated sampling moments and might not reflect the real N status of each site. On the other hand, seaweeds may offer a more reliable picture of what might be happening in the surrounding environment. The tissue nitrogen content of *G. chilensis* after 2 months in culture revealed the different availability of this nutrient in the experimental areas.

Our results showed that the long-line placed 800 m away from the salmon pens had the best performance both in productivity and nitrogen removal. The monthly average N removal by *G. chilensis* cultivated at this site reached values of 9.3 g m^{-1} of culture line. Troell et al. (1997) found similar N reduction capacity for a *G. chilensis* system placed 10 m distant from a farm producing 287 tonnes of salmon per year. This suggests that our system would have improved N reduction capacity with decreasing distance to the salmon farm. Nevertheless, we got better results at 800 m rather than at 100 m distance from the fish cages. The lower levels of productivity at 100 m were probably associated with the higher occurrence of epiphytes and generally less clean aspect of the seaweeds grown at the closest distance (pers. observ.). These observations imply that aside from distance, other features of the water column (e.g. suspended organic matter) must be taken into account when making decisions on the cultivation system location. Considering the 9.3 g N m^{-1} removal potential of the long-line placed at 800 m, we were able to predict that the minimal area for a *Gracilaria* biofiltration system effective in reducing the N loads can be over 100 ha surrounding a 1500 tonnes salmon farm. This information is essential for the design of an efficient IMTA system.

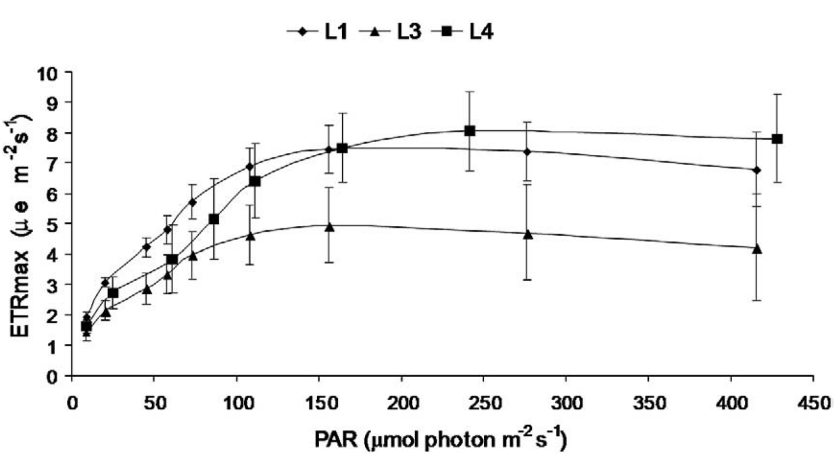

Fig. 7. Rapid light curves obtained in laboratory for *Gracilaria chilensis* grown close to the salmon cages (L1) and away from them (L3 — suspended culture and L4 — bottom culture). Mean ± SD, n = 10.

Considering this cultivation area (100 ha), the installation of 25 lines per ha, and the attachment of metal frames in order to optimize the surface area available for seaweed growing (see Halling et al., 2005), this seaweed biofiltration system could remove around 9300 kg of dissolved N per month during summer conditions. For the colder months, with lower values of seaweed production, the nitrogen removal of this system will drop to values around 1990 kg month^{-1}. A production cycle for salmon in Chile is around 12 months (residency time in cages before harvest). If we consider an annual dissolved N release of 43.35 kg per tonne of fish produced (Mente et al., 2006), the N load of a 1500 tonnes salmon farm would be 65 tonnes N per year. Combining 6 months of low seaweed production with another semester of high productivity, these figures indicate that ca. 100 ha of seaweed culture could biofiltrate up to 100% the N loads of a salmon farm with 1 ha, 2 cages and 1500 tonnes of fish produced.

One of the ways proposed to reduce the area required for the seaweed component in IMTA systems, is to increase the seaweed productivity by culturing different algal species with different optimal depths (Buschmann et al., 2008c). Other developments in this line of thinking are necessary to achieve an efficient use of space by IMTA systems.

Many studies have evaluated the benthic impact of the suspended matter released by salmon aquaculture (see recent reviews: Holmer et al., 2008; Rensel et al., 2006). However, the relative importance of dissolved nitrogen inputs by these fish farms remains under debate, since it is commonly thought that the downstream flow of this nutrient reduces its latent concentrations to levels below environmental threat (see Sellner et al., 2003 for review). Our results provide the information that the required assimilation surface to reduce the dissolved nitrogen input of a 1 ha commercial salmon farm would be over 100 ha, under natural conditions. This reflects the impact level that one single salmon farm might have in terms of nitrogen release. Taking into account the high density of salmon farms established in southern Chile and, even more, their localization in relatively enclosed bays, it is necessary that the environmental stakeholders increase their focus on dissolved nutrient release. After establishing the required area and biofiltration performance of the IMTA's seaweed component, it becomes necessary to economically quantify this environmental service. The obligation to include environmental costs in companies' budget might increase their sensitivity to this problem (Neori et al., 2007). The integration of *G. chilensis* with fish farms as already been reported to reduce these costs and even increase the companies' profitability (Buschmann et al., 2001a; Chopin et al., 2001). Considering the numbers determined in the present study, we confirm that the application of an IMTA approach in Chile would greatly benefit the entire aquaculture sector.

The comparison between the two *G. chilensis* cultivation approaches showed that the long-line system is the more advantageous. Growth rates of the long-line culture were consistently higher than the traditional bottom culture method used in southern Chile (Buschmann et al., 1995). It showed similar growth rates and productivity reported in other studies (Halling et al., 2005; Troell et al., 1997). The *G. chilensis*'s bottom cultivation unit set in the mud-flat at Metri Bay presented high levels of variation on the production levels (from 0.4 to 2 kg m^{-1} month^{-1}) and severe material losses caused by the instability of the sandy bottoms. The place where the cultivation unit was installed is ca. 600 m away from a small scale salmon farm and a mussel farm. Although no current measurements were taken, due to the geographical features of the area, it seems likely that this is also a nitrogen impacted site. Probably this explains the high values of biomass production encountered; nevertheless, the biomass variability at this site and the culture method applied during the experiment makes it a fallible system with lower warranties of stability on biomass production.

In order to optimize seaweed production, it is highly recommended to determine the physiological status of the algae in culture. Through chlorophyll fluorescence we monitored the photosynthetic performance of *G. chilensis* at each cultivation site. Photosynthetic activity normally decreases at midday, immediately after the irradiance peak, and recovers during the afternoon (Hanelt et al., 1993). When this recovery is successful it is said that macroalgae experienced a dynamic photoinhibition (Figueroa and Gómez, 2001). Our results of *in situ* Fv/Fm showed that *G. chilensis*, at all cultivation sites, recovered rapidly from any photoinhibition after peak light levels. The daily cycles of yield (Φ_{PSII}) and rETR values were similar to other reports; decreasing after the irradiance peak with recovery to morning initial values at all sites (Gómez et al., 2005). *G. chilensis* has the ability to adapt to a wide range of PAR and UV levels (Buschmann et al., 2008c; Gómez et al., 2004, 2005). Midday summer levels of UV-B in southern Chile are high enough to produce stress to intertidal macroalgae (Huovinen et al., 2006). We did not record the UV intensity, but Gómez et al. (2005) reported that this radiation type gets absorbed in the first 30 cm of the water column, suggesting that it may not be a relevant factor in our long-line culture. We found that ca. 25% of the incident irradiance reaches the cultivation depth of 1 m with average PAR values of 300 µmol m^{-2} s^{-1}. The obtained values for E_k were lower than the ones found in other studies (Buschmann et al., 2008c; Gómez et al., 2004) and considered within the range described for shade-adapted species (Gómez et al., 2005). Our study's E_k values together with the measured PAR levels suggest that *G. chilensis* was undergoing photosynthesis under light saturated conditions during most of the day, confirming 1 m depth as ideal for this species cultivation. In relation to the bottom culture method, it seems that the high amplitude tidal variation (up to 7 m in this region) implying lower PAR levels reaching the seaweed, might explain the highest E_k values obtained for *G. chilensis* cultivated at Metri (L4). This factor together with the occasional sediment burial (pers. observ.) could be the cause to the variation in productivity observed at that site.

In addition to light, nitrogen availability also affected the photosynthetic performance of *G. chilensis*. The proximity to the salmon farm seems to have mitigated the decrease of the photosynthetic activity after the midday irradiance peak. *G. chilensis* cultivated next to the salmon farms (including the bottom culture) was less affected by the irradiation peak. The differences found for α and the maximal values of photosynthesis (as ETR_{max}) are in accordance with the photosynthetic activity measured *in situ* at the cultivation sites. Consequently, *G. chilensis* presented a better photosynthetic performance at the sites where it attained the highest productivity and nitrogen removal capacity. Other studies related the nitrogen availability to photosynthetic performance of seaweeds in culture. Mata et al. (2006) found a decrease in Fv/Fm from *Asparagopsis armata* cultivated under low nitrogen treatments. The photoprotection of red algae has also been related to the nitrogen availability. The accumulation of ultraviolet-screen substances, mycosporine-like amino acids (MAAs) might increase under N enriched conditions (Figueroa et al., 2008; Huovinen et al., 2006; Korbee et al., 2005; Peinado et al., 2004).

More studies, with extended experimental periods together with controlled laboratory experiments testing a wide range of N concentrations, are still needed. These would help to validate photosynthetic performance as a quick and robust measure to predict N stress episodes in large scale seaweed farming. Nonetheless, the relation between these two factors is established and it is clear that seaweeds grown in suspended lines close to the salmon cages take advantage of the extra source of nitrogen to improve their photosynthetic performance, reaching higher production levels.

5. Conclusions

The interest in *G. chilensis* biomass production is likely to keep increasing. Agar industry will continue to be the basis for the exploitation of this natural resource; nevertheless, promising new market applications for *Gracilaria* species, such as the extraction of MAAs for sunblock cosmetics (Cardozo et al., 2007), or as alternative protein source in fish feeds (Valente et al., 2006) have come to light. It is thus crucial to pay a closer look to the management of this resource and improve its profitability. Cultivation of *G. chilensis* in long-lines within an IMTA system was proven more advantageous than in the traditional bottom culture; this seaweed's productivity and physiological performance were greatly improved.

Production and profitability are normally the main factors taken into account before making an economic investment. Simple calculations based on this work and on the current commercial value of *G. chilensis* biomass (Buschmann et al., 2008b) point to an expected income of nearly 6 times more the initial investment necessary for a 100 ha *G. chilensis* seaweed farm. The maintenance of an environmental balance, although still not legislated and with no money costs, is already being used as a marketing approach by several companies and is currently under public debate (www.contralacorriente.cl, www.salmonchile.cl).

This study confirms the biofiltration efficiency of *G. chilensis* and suggests that a 100 ha *G. chilensis* farm will effectively (ca. 100%) reduce the inorganic N inputs of a 1500 tonnes salmon farm. Nevertheless, in order to estimate the importance of some factors that may lower this system's performance (e.g. horizontal/vertical nutrient dilution), it is necessary to establish a full scale seaweed farm. In a perfect scenario, the inclusion of organic filter organisms (e.g. mussel) would complete this IMTA system.

This information is now available for the local fish farms and might help in the development of environmental regulations.

Our main objectives were achieved, demonstrating the net benefits from a *Gracilaria*-based IMTA system, potentially available for traditional seaweed farmers as well as to fish farmers.

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References

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.
Barton, J.R., 1997. Environment, sustainability and regulation in commercial aquaculture: the case of Chilean salmonid production. Geoforum 28, 313–328.
Beer, S., Larsson, C., Poryan, O., Axelsson, L., 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by Pulse Amplitude Modulated (PAM) fluorometry. Eur. J. Phycol. 35, 69–74.
Blouin, N., Xiugeng, F., Peng, J., Yarish, C., Brawley, S.H., 2007. Seeding nets with neutral spores of the red alga *Porphyra umbilicalis* (L.) Kützing for use in Integrated Multi-Trophic Aquaculture (IMTA). Aquaculture 270, 77–91.
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. Aquac. Eng. 13, 283–300.
Buschmann, A.H., Westermeier, R., Retamales, C.A., 1995. Cultivation of *Gracilaria* in the sea-bottom in southern Chile: a review. J. Appl. Phycol. 7, 291–301.
Buschmann, A.H., Troell, M., Kautsky, N., Kautsky, L., 1996. Integrated tank cultivation of salmonids and *Gracilaria chilensis* (Rhodophyta). Hydrobiologia 326/327, 75–82.
Buschmann, A.H., Troell, M., Kautsky, N., 2001a. Integrated algal farming: a review. Cah. Biol. Mar. 42, 83–90.
Buschmann, A.H., Correa, J., Westermeier, R., Hernández-González, M., Norambuena, R., 2001b. Cultivation of red algae in Chile: a review. Aquaculture 194, 203–220.
Buschmann, A.H., Riquelme, VA, Hernández-González, M.C., Varela, D.A., Jiménez, J.E., Henríquez, L., Vergara, P.A., Guíñez, R., Filún, L, 2006. A review of the impacts of salmon farming on marine coastal ecosystems in the southeast Pacific. ICES J. Mar. Sci. 63, 1338–1345.
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. Vol. [3] of Encyclopedia of Ecology, vol. 5. Elsevier, Oxford, pp. 2211–2217.
Buschmann, A.H., Hernández-González, Varela, D.A., 2008b. Seaweed future cultivation in Chile: perspectives and challenges. Int. J. Environ. Pollut. 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. J Appl. Phycol. 20, 571–577.
Cabello-Pasini, A., Figueroa, F.L., 2005. Effect of nitrate concentration on the relationship between photosynthetic oxygen evolution and electron transport rate in *Ulva rigida* (Chlorophyta). J. Phycol. 41, 1169–1177.
Cabello-Pasini, A., Aguirre-von-Wobeser, E., Figueroa, F.L., 2000. Photoinhibition of photosynthesis in *Macrocystis pyrifera* (Phaeophyceae), *Chondrus crispus* (Rhodophyceae) and *Ulva lactuca* (Chlorophyceae) in outdoor culture systems. J. Photochem. Photobiol., B Biol. 57, 169–178.
Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcão, V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P., Pinto, E., 2007. Metabolites from algae with economical impact. Comp. Biochem. Physiol., Part C 146, 60–78.
Chopin, T., Buschmann, A.H., Halling, C., Troell, M., Kautsky, N., Neori, A., Kraemer, G.P., Zertuche-González, J.A., Yarish, C., Neefus, C., 2001. Integrating seaweeds into aquaculture systems: a key towards sustainability. J. Phycol. 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. Vol. [3] of Encyclopedia of Ecology, vol. 5. Elsevier, Oxford, pp. 2463–2475.
Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270, 1–14.
Davison, I.R., 1991. Environmental effects on algal photosynthesis: temperature. J. Phycol. 27, 2–8.
D'Elia, C.F., DeBoer, J.A., 1978. Nutritional studies of two red algae: II. Kinetics of ammonium and nitrate uptake. J. Phycol. 14, 266–272.
Figueroa, F.L., Gómez, I., 2001. Photosynthetic acclimation to solar UV radiation of marine red algae from the grass temperate coast of southern Spain. A review. J. Appl. Phycol. 13, 235–248.
Figueroa, F.L., Santos, R., Conde-Álvarez, R., Mata, L., Pinchetti, J.L.G.ó.m.e.z., Matos, J., Huovinen, P., Schuenhoff, A., Silva, J., 2006. Bot. Mar. 49, 275–282.
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*. J. World Aquac. Soc. 39, 692–699.
Flores-Aguilar, R.A., Gutierrez, A., Ellwanger, A., Searcy-Bernal, R., 2007. Development and current status of abalone aquaculture in Chile. J. Shellfish Res. 26, 705–711.
Genty, B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990, 87–92.
Gómez, I., Figueroa, F.L., Ulloa, N., Morales, V., Lovengreen, C., Huovinen, P., Hess, S., 2004. Photosynthesis in 18 intertidal macroalgae from Southern Chile. Mar. Ecol., Prog. Ser. 270, 103–116.
Gómez, I., Figueroa, F.L., Huovinen, P., Ulloa, N., Morales, V., 2005. Photosynthesis of the red alga *Gracilaria chilensis* under natural solar radiation in an estuary in southern Chile. Aquaculture 244, 369–382.
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. Aquac. Int. 13, 409–422.
Hanelt, D., Huppertz, K., Nultsch, W., 1993. Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in laboratory and in the field. Mar. Ecol., Prog. Ser. 97, 31–37.
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.
Henley, W.J., Levavasseur, G., Franklin, L.A., Osmond, C.B., Ramus, J., 1991. Photoacclimation and photoinhibition in *Ulva rotundata* as influenced by nitrogen availability. Planta 184, 235–243.
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.
Huovinen, P., Matos, J., Sousa-Pinto, I., Figueroa, F.L., 2006. The role of ammonium in photoprotection against high irradiance in the Mediterranean red alga *Grateloupia lanceola*. Aquat. Bot. 84, 308–316.
Jassby, A.D., Platt, T., 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21, 540–547.
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.
Korbee, N., Huovinen, P., Figueroa, F.L., Aguilera, J., Karsten, U., 2005. Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two *Porphyra* species (Bangiales, Rhodophyta). Mar. Biol. 146, 645–654.
Kraemer, G.P., Yarish, C., 1999. A preliminary comparison of the mariculture potential of *Porphyra purpurea* and *Porphyra umbilicalis*. J. Appl. Phycol. 11, 473–477.
Kraemer, G.P., Carmona, R., Chopin, T., Yarish, C., 2002. Use of photosynthesis measurements in the choice of algal species for bioremediation. In: Van Patten, P. (Ed.), Proc. of the Long Island Sound Research Conference, Nov. 17–18, 2000, Stamford, CT. Connecticut Sea Grant College Program. ISBN: 1-878301-06-3, pp. 113–117.
Lamote, M., Johnson, L.E., Lemoine, Y., 2007. Interspecific differences in the response of juvenile stages to physical stress: fluorometric responses of fucoid embryos to variation in meteorological conditions. J. Phycol. 43, 1164–1176.

Longstaff, B.J., Kildea, T., Runcie, J.W., Cheshire, A., Dennison, W.C., Hurd, C., Kana, T., Raven, J.A., Larkum, A.W.D., 2002. An *in situ* study of photosynthetic oxygen exchange and electron transport rate in the marine macroalga *Ulva lactuca* (Chlorophyta). Photosynth. Res. 74, 281–293.
López, J., 2008. Salmon and Trout report. Infopesca. FAO Globe Fish.
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.
McVey, J.P., Stickney, R., Yarish, C., Chopin, T., 2002. Aquatic polyculture and balanced ecosystem management: new paradigms for seafood production. In: Stickney, R.R., McVey, J.P. (Eds.), Responsible Aquaculture. CAB International, Oxon, U.K., pp. 91–104.
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. Aquac. Int. 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.
Neori, A., Shpigel, M., Ben-Ezra, D., 2000. A 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.
Parkhill, J.P., Maillet, G., Cullen, J., 2001. Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J. Phycol. 37, 517–529.
Peinado, N.K., Díaz, R.T.A., Figueroa, F.L., Helbling, E.W., 2004. Ammonium and UV radiation stimulate the accumulation of Mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. J. Phycol. 40, 248–259.
Pereira, R., Yarish, C., Sousa-Pinto, I., 2006. The influence of stocking density, light and temperature on the growth, production and nutrient removal capacity of *Porphyra dioica* (Bangiales, Rhodophyta). Aquaculture 252, 66–78.
Rensel, J.E., Buschmann, A.H., Chopin, T., Chung, I.K., Grant, J., Helsley, C.E., Kiefer, D.A., Langan, R., Newell, R.I.E., Rawson, M., Sowles, J.W., McVey, J.P., and Yarish, C. 2006. Ecosystem based management: models and mariculture. Rensal, J. (Editor). Chapter 10, Pages 207–220 in J.P. McVey, C-S. Lee, and P.J. O'Bryen, editors. "Aquaculture and Ecosystems: An Integrated Coastal and Ocean Management Approach." The World Aquaculture Society, Baton Rouge, Louisiana, 70803. United States.
Rodrigueza, M.R.C., Montaño, M.N.E., 2007. Bioremediation potential of three carrageenophytes cultivated in tanks with seawater from fish farms. J. Appl. Phycol. 19, 755–762.
Sahoo, D., Yarish, C., 2005. Mariculture of seaweeds. In: Andersen, R. (Ed.), Phycological Methods: Algal Culturing Techniques. Academic Press, New York, pp. 219–237.
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.
Santelices, B., Ugarte, R., 1987. Production of Chilean *Gracilaria*: problems and perspectives. Hydrobiologia 152, 295–299.
Sellner, K.G., Doucette, G.J., Kirkpatrick, G.J., 2003. Harmful algal blooms: causes, impacts and detection. J. Ind. Microbiol. Biotech. 30, 383–409.
Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799–801.
Strickland, J.D.H., Parsons, T.R., 1972. A Practical Handbook of Sea-water Analysis, 2nd ed. J. Fish. Res. Bd. Canada, vol. 167. 311 pp.
Tacon, A.G.J., Halwart, M., 2007. Cage aquaculture: a global overview. In: Halwart, M., Soto, D., Arthur, J.R. (Eds.), Cage aquaculture — Regional Reviews and Global Overview, pp. 1–16. FAO Fisheries Technical Paper. No. 498. FAO, Rome. 241 pp.
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.
Troell, M., Robertson-Andersson, D., Anderson, R.J., Bolton, J.J., Maneveldt, G., Halling, C., Probyn, T., 2006. Abalone farming in South Africa: an overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture 257, 266–281.
Underwood, A.J., 1997. Experiments in Ecology. Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, Cambridge, UK. 504 pp.
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.
World Wildlife Fund, 2007. (June 22) Salmon Farming Threatens Chile's Patagonian Lakes. ScienceDaily. Retrieved October 19, 2008, from http://www.sciencedaily.com /releases/2007/06/070621202901.html.
Yarish, C. and Pereira, R. 2008. Mass production of marine macroalgae. *In* Sven Erik Jørgensen and Brian D. Fath (Editor-in-Chief), Ecological Engineering. Vol. [3] of Encyclopedia of Ecology, 5 vols. pp. 2236–2247. Oxford: Elsevier.

Web references

http://www.contralacorriente.cl/.
http://www.salmonchile.cl/frontend/seccion.asp?contid=473&secid=6&secoldid=6&sbsecid=141&pag=1.