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Research Article

Nutrient uptake efficiency of *Gracilaria chilensis* and *Ulva lactuca* in an IMTA system with the red abalone *Haliotis rufescens*

Juan Macchiavello[1] & Cristian Bulboa[2]
[1]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte
P.O. Box 117, Coquimbo, Chile
[2]Departamento de Ecología y Biodiversidad, Facultad de Ecología y Recursos Naturales
Universidad Andres Bello, Avda. República 440, Santiago, Chile

ABSTRACT. The current study examined the nutrient uptake efficiency of *Ulva lactuca* and *Gracilaria chilensis* cultivated in tanks associated with the wastewater of a land-based abalone culture. The experiments evaluated different seaweed stocking densities (1200, 1900, 2600, and 3200 g m^{-2}) and water exchange rates (60, 80, 125, and 250 L h^{-1}). The results show that both *U. lactuca* and *G. chilensis* were efficient in capturing and removing all of the inorganic nutrients originating from the abalone cultivation for all of the tested conditions. Furthermore, an annual experiment was performed with *U. lactuca*, cultivated at a stocking density of 1900 g m^{-2} and at a water exchanged rate of 125 L h^{-1}, in order to evaluate seasonal changes in the nutrient uptake efficiency, productivity, and growth rate associated with the wastewater of a land-based abalone culture. The results confirmed high uptake efficiency during the entire year, equivalent to a 100% removal of the NH_4, NO_3, and PO_4 produced by the land-based abalone culture. The growth rate and productivity of *U. lactuca* presented a marked seasonality, increasing from fall until summer and varying from 0.5 ± 0.2% to 2.6 ± 0.2% d^{-1} and 10 ± 6.1% to 73.6 ± 8.4% g m^{-2} d^{-1} for sustainable growth rate and productivity, respectively. We conclude that there is sufficient evidence that demonstrates the high possibility of changing the traditional monoculture system of abalone in Chile, to a sustainable integrated multi-trophic aquaculture system, generating positive environmental externalities, including the use of *U. lactuca* as a biofiltration unit.

Keywords: *Haliotis rufescens*, abalone, multitrophic, seaweeds, *Gracilaria*, *Ulva*, aquaculture, Chile.

Eficiencia de absorción de nutrientes de *Gracilaria chilensis* y *Ulva lactuca* en un sistema multitrófico integrado con el abalón rojo *Haliotis rufescens*

RESUMEN. Se analizó la eficiencia de captación de nutrientes de *Ulva lactuca* y *Gracilaria chilensis*, cultivadas en estanques asociados a aguas de desecho proveniente de un cultivo del abalón rojo *Haliotis rufescens*. Los experimentos consideraron evaluar diferentes densidades de cultivo de algas (1200, 1900, 2600 and 3200 g m^{-2}) y tasas de recambio de agua (60, 80, 125 y 250 L h^{-1}). Los resultados mostraron que tanto *U. lactuca* como *G. chilensis* fueron eficientes en la captación de nutrientes inorgánicos provenientes del cultivo de abalón, en todas las condiciones probadas, con remoción total de los nutrientes aportados por el cultivo. De la misma forma se realizó un experimento anual con *U. lactuca*, siendo cultivada con densidad de 1900 g m^{-2} y tasa de recambio de agua de 125 L h^{-1} para evaluar cambios estacionales en la eficiencia de captación de nutrientes, así como en la productividad y tasa de crecimiento de *U. lactuca*. Los resultados confirmaron una alta eficiencia de captación de *U. lactuca* durante todo el año, equivalente a la remoción del 100% del NH_4, NO_3 y PO_4 aportado por el cultivo de abalón. La tasa de crecimiento y la productividad de *U. lactuca* presentó una marcada estacionalidad, incrementando de otoño al verano, variando de 0,5 ± 0,2 a 2,6 ± 0,2% d^{-1} y 10 ± 6,1 a 73,6 ± 8,4 g m^{-2} d^{-1}, respectivamente. Se concluye que existen antecedentes suficientes que demuestran que es altamente posible cambiar la actividad tradicional de cultivo de abalón por un sistema integrado multitrófico, alcanzando externalidades ambientales positivas que incluyen *U. lactuca* como unidad de biofiltración.

Palabras clave: *Haliotis rufescens*, abalón, algas, multitrófico, *Gracilaria*, *Ulva*, acuicultura, Chile.

Corresponding author: Cristian Bulboa (cbulboa@unab.cl)

INTRODUCTION

The red abalone *Haliotis rufescens* (Swainson, 1822) is a non-native species of the Chilean coast which was introduced in 1977 for laboratory analysis (Godoy & Jerez, 1998). Since then, diverse investigations have been performed which have permitted the development of cultivating this species, thus giving rise to the rapid growth of commercial activities since the 90's. In the year 2006, Chile positioned itself as the fifth largest producer of abalones in the world with 304 ton. In 2009, 886 ton were produced (SERNAPESCA, 2009), demonstrating increased production and an industry that finds itself in expansion, with currently twenty-five companies related to the production of abalone in Chile (Flores-Aguilar *et al.*, 2007). The majority of these installations are land-based cultures principally located in the northern region of the country and which take on seed production as well as the adult growth. Conversely, cultivation in the south is predominately sea-based, and, in some cases, provided with seeds produced in the north (Flores-Aguilar *et al.*, 2007). In this way, there is a high expected potential for increasing abalone production in the future, based on cultivation site availability in the south of Chile as well as the expansion of the limits for sea-based cultivation in the north. This situation implies an increase of installations to support the rise in demand for seeds and supplies, bringing a greater pressure to the environment and possible conflicts, especially concerning waste-water which is currently discharged into the sea. This situation has been widely documented for different cultivated species of the world, where the rapid expansion of aquaculture has translated into a growing interest for scientists, industrialists, and governments alike in understanding and minimizing the impacts that these productive activities could have on the environment (Troell *et al.*, 2006). Special concern is given to the eutrophication process, where an excessive quantity of N, P, and CO_2 and lowered levels of dissolved O_2, a product of intense animal cultivation in tanks, ponds, and coastal waters, has caused serious problems in coastal waters of the world (Fei, 2004; Yokoyama & Ishihi, 2010). Arising from this, the high effectiveness of using seaweeds for the treatment of wastewater produced by marine animal cultivation has been documented through the use of integrated cultures (Chopin *et al.*, 2001; Fei, 2004; Bolton *et al.*, 2009; Abreu *et al.*, 2011). The Integrated Multi-Trophic Aquaculture (IMTA) model (Neori *et al.*, 2004), couples species of distinct trophic or nutritional levels into the same system (FAO, 2009). For example, seaweeds, as primary producers, function as a biofilter by removing CO_2 and nutrient wastes, like dissolved nitrogen and phosphorus, from seawater and thus reduce nutrient discharge into the environment (Troell *et al.*, 2003; Nobre *et al.*, 2010). Additionally, the high nutrient uptake capacity of seaweed promotes its growth (Troell *et al.*, 2006), generating potentially valuable biomass that can be used as a protein-enriched feed for other species or in human food, phycocolloids, cosmetics, and the medicine market, among other uses, thus increasing and diversifying the income of an IMTA system (Neori, 2008).

Until now, the principal consequence of abalone cultivation in Chile has been the harvesting of brown algae from natural marine beds for feed, causing an overexploitation of this resource (Vásquez, 2008). However, the successful development of *Macrocystis* farming (Westermeir *et al.*, 2006; Macchiavello *et al.*, 2010), currently allows abalone producers to count on fresh biomass. Nonetheless, other problems associated with abalone cultivation have not been noted, and the potential growth of farms and their concentration mainly in bay restricted areas, which possess multiple uses, makes necessary a sustainable plan for expansion in order to avoid environmental conflicts. In this same line, diverse studies have already address the environmental, social, and economical advantages of abalone-seaweed integrated cultivation (Troell *et al.*, 2006; Robertson-Andersson *et al.*, 2008; Bolton *et al.*, 2009; Nobre *et al.*, 2010). Thus, consolidation of abalone farming as an environmentally responsible aquaculture process is highly necessary, and implies an enormous opportunity for the industry to be recognized as one with positive environmental consequences, an especially important goal in Chile, where aquaculture is closely related with salmon farming and its negative environmental impacts of the last decade (Buschmann *et al.*, 2009; FAO, 2009).

In this paper, we evaluated the temporal effectiveness of integrating a land-based culture of red abalone *H. rufescens* and two well-known algae of the Chilean coast, *Ulva lactuca* and *Gracilaria chilensis*, which have been studied in integrated systems, demonstrating high nutrient uptake efficiency and biomass production, and have been mentioned as the most suitable candidates for IMTAs (Neori *et al.*, 2000; Abreu *et al.*, 2009; Bolton *et al.*, 2009). For our evaluation, the algae stocking density and water exchange rate were tested to establish the dissolved nutrient uptake efficiency of *U. lactuca* and *G. chilensis*, in addition to measuring the effects on growth rate and productivity of *U. lactuca*. It was expected that an adequate combination of these factors could maintain optimum efficiency for the integrated culture throughout the year and generate evidence to propose a base-line move from the traditional, monoculture of abalone in Chile, to a sustainable IMTA system.

MATERIALS AND METHODS

The experiments were performed at the Centro de Producción de Semillas de Abalón of the Universidad Católica del Norte, Coquimbo, Chile, located in the bahía La Herradura de Guayacán (30°S, 71°W). The center cultivates approximately 1200 adult organisms and 50000 seeds of red abalone *H. rufescens* (10 to 30 mm) with a total seawater flow of 100000 L h^{-1}. The abalones are fed once per week with fresh seaweed, such as *Macrocystis*, *Lessonia*, and *Ulva.* Seawater is captured from the bay through filtered (5 mm) subsea tubes and is subsequently filtered to 50 µm before being introduced into the culture.

Experimental setup

Ulva lactuca and *Gracilaria chilensis* were collected from the bahía La Herradura de Guayacán and cultivated using water originating from the abalone culture, which was accumulated in a tank of 5000 L providing a constant flow of seawater to the cultivated algae. These cultures consisted of twenty-four white fiberglass tanks (twelve for each algae) with a volume of 250 L (100x50x50 cm) and a constant aeration, maintained in outdoor conditions. In these installations, we performed the experiments (Fig. 1). The abiotic conditions during the experiment 1 and 2 were temperature (15 ± 2°C), pH (7.9 ± 1), salinity (35 ± 1‰), and photon flux density (794 ± 190 µmol photon m^{-2} s^{-1}). All these parameters were measured daily.

Experiment 1: Seaweed stocking density

For the experiment, four stocking densities of 1200, 1900, 2600, and 3200 g m^{-2} for *G. chilensis* and *U. lactuca* were used. Three culture tanks, as described above (Fig. 1), were used for both *G. chilensis* and *U. lactuca* for each stocking density. In all treatments, the water exchange rate was maintained in a continuous seawater flow at 80 L h^{-1}. The algae were cultivated for 60 days, and every 15 days, biomass was harvested to maintain the initial stocking density. Quantitative analysis was performed every 15 days for ammonium, nitrate, and phosphate. For that, three water samples (100 mL) were taken in the seawater inflow, abalone outflow and seaweed outflow (Fig. 1). The analyses were performed using the Strickland & Parson (1972) method, and the results were measured as µmol L^{-1} with a subsequent expression as nutrient uptake efficiency (%), using the following formula:

$$\text{Nutrient uptake efficiency} = 100 - (100 \times C_{So}/C_{ao})$$

where: C_{so}= seaweed outflow concentration, and C_{ao}= abalone outflow concentration.

Experiment 2: Water Exchange Rate (WER)

Four WERs of 60, 80, 125 and 250 L h^{-1} were used to test the nutrient uptake of *G. chilensis* and *U. lactuca*. For this experiment, the culture density was maintained at a constant of 1900 g m^{-2}. The algae were cultivated for 60 days, and every 15 days, biomass was harvested to maintain the initial stocking density. The water sampling and analyses were performed and expressed following the same procedures as outlined in Experiment 1.

Experiment 3: Seasonal nutrient uptake efficiency, specific growth rate, and productivity of *U. lactuca*

Considering the results of the previous experiments, the seasonal experiment chose the species *U. lactuca* as a model, with a culture density of 1900 g m^{-2} and a WER of 125 L h^{-1}. This species was chosen for its lack of epiphytism in comparison with *G. chilensis* during experiments 1 and 2. From these same experiments, algae density and seawater flow were chosen based on the best uptake efficiency for NH_4, NO_3, and PO_4 during the cultivation of *U. lactuca*. Algae were cultivated for one year in twelve tanks of 250 L, as described in experiments 1 and 2. Temperature in culture tanks was registered daily. The algae were weighed monthly and the biomass surplus was withdrawn to maintain initial biomass. For each season, productivity (g m^{-2} d^{-1}) and specific growth rate (SGR) were calculated using the following formula:

$$SGR(\%d^{-1}) = \frac{Ln(Wf) - Ln(Wi)}{T}100$$

where, Wf = final wet weight; Wi = initial wet weight; T = days

Statistical analysis

The homocedasticity and normality of all results were verified. A multivariate ANOVA analysis was conducted for each nutrient source (NH_4, NO_3, and PO_4) of the experiments 1 and 2 to evaluate the effect of water exchange rate and stock density on uptake efficiency of *G. chilensis* and *U. lactuca*, respectively. For SGR and productivity, a one-way ANOVA analysis was performed in order to evaluate differences among seasons. A posterior Tukey's test was used when the treatments showed significant differences ($P < 0.05$).

RESULTS

During the development of the experiments, the abalone culture constantly supplied nutrients to the seawater. Table 1 reviews the active contribution of nutrients that the abalone culture provided (abalone outflow) and the effect of seaweed culture removing nutrients (seaweed outflow).

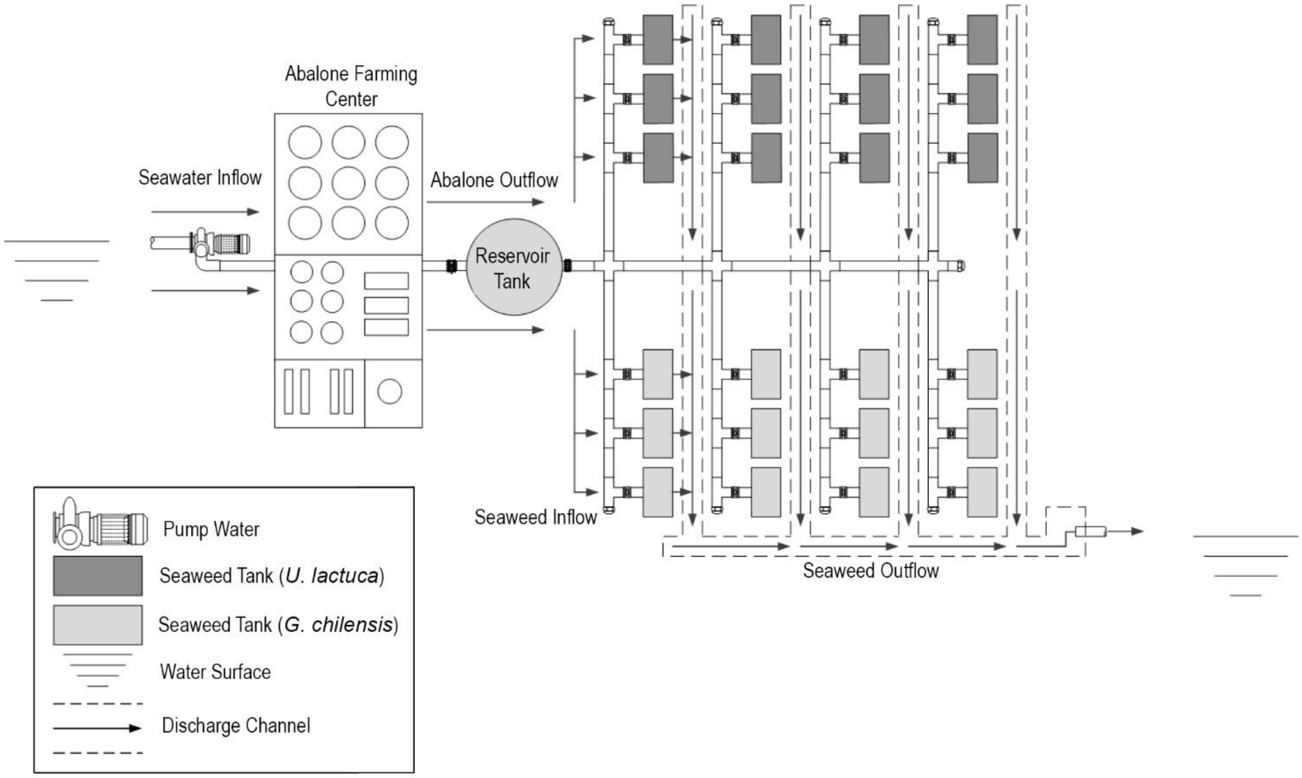

Figure 1. Schematic representation of the seaweed biofiltration system. Arrows indicate water flows.

Experiment 1: Stocking density

The uptake efficiency of NH_4 was different between both macroalgae species (ANOVA, F = 28.973, $P < 0.05$) and stocking densities tested (ANOVA, F = 9.940, $P < 0.05$). Figure 2 shows that *G. chilensis* was able to uptake 100% of NH_4 for all stocking densities tested. However, in the case of *U. lactuca*, the NH_4 uptake efficiency was 100% only at the lowest densities (1200 and 1900 g m^{-2}), while at 2600 and 3200 g m^{-2} the NH_4 uptake efficiency decreased significantly (Tukey, $P < 0.05$), reaching 89 and 86%, respectively. For both species, NO_3 uptake efficiency was less than 100% for all stocking densities (Fig. 2). However, no statistical differences were found between the tested species (ANOVA, F = 0.301, $P > 0.05$) and stocking densities (ANOVA, F = 1.431, $P > 0.05$). For *G. chilensis*, values ranged from 65 to 88% at 1900 and 3600 g m^{-2}, respectively. *U. lactuca* showed low variation for NO_3 uptake efficiency, with the highest uptake efficiency registered at 1200 g m^{-2} (83%) and the lowest at 3200 g m^{-2} (65%). Statistical differences for PO_4 uptake efficiency were registered for different stocking densities (ANOVA, F = 3.757, $P < 0.05$). These were produced by the highest and lowest densities registered for *U. lactuca* (Tukey, $P < 0$ .05), with maximum PO_4 uptake efficiency at 1900 and 1200 g m^{-2} (65 and 64%, respectively) and a minimum at 2600 and 3200 g m^{-2} (13%). *G. chilensis* PO_4 uptake efficiency did not show significant differences (Tukey, $P > 0.05$) among stocking densities, and ranged from 38 to 24% at 2600 and 1200 g m^{-2}, respectively (Fig. 2).

Experiment 2: Water Exchange Rate (WER)

Figure 3 shows that uptake efficiency of NH_4, NO_3, and PO_4 was different among the WERs used (ANOVA, F = 114.077, $P < 0.05$) and among the species (ANOVA, F = 73.987, $P < 0.05$).

For *G. chilensis*, high NH_4 uptake efficiency was reached at both 80 and 125 L h^{-1} when compared to uptake at 60 and 250 L h^{-1} (Tukey, $P < 0.05$). The highest NH_4 efficiency uptake for this species was registered at 125 L h^{-1} (96 ± 2%) and the lowest was at 60 L h^{-1} (59 ± 3%). A similar situation was observed for the NH_4 uptake efficiency of *U. lactuca*, where the highest uptake, was registered at 80 L h^{-1} and the lowest, at 250 L h^{-1} (Tukey, $P < 0$ .05) (Fig. 3).

NO_3 uptake efficiency of *G. chilensis* showed differences between the lowest efficiencies at 125 and 250 L h^{-1} (66 ± 4 and 67 ± 4%, respectively) and highest efficiency at 80 L h^{-1} (85 ± 2%) (Tukey, $P < 0.05$). For

Table 1. Nutrient concentration (NH_4, NO_3 and PO_4) registered in seawater inflow, abalone outflow and seaweed outflow in each of the experiments. Data: Mean ± Standar deviation.

Experiment	Species	Treatment	Effluent	NH_4 µmol L^{-1}	NO_3 µmol L^{-1}	PO_4 µmol L^{-1}
			Seawater inflow	0.176 ± 0.034	6.43 ± 1.19	1.16 ± 0.22
			Abalone outflow	0.889 ± 0.112	7.21 ± 0.47	0.97 ± 0.26
1	*Gracilaria chilensis*	1200 g m^{-2}	Seaweed outflow	0.000	2.91 ± 0.78	0.70 ± 0.19
		1900 g m^{-2}		0.000	3.45 ± 0.51	0.75 ± 0.11
		2600 g m^{-2}		0.000	2.96 ± 0.20	0.65 ± 0.26
		3200 g m^{-2}		0.000	3.01 ± 0.18	0.66 ± 0.20
	Ulva lactuca	1200 g m^{-2}	Seaweed outflow	0.000	2.01 ± 0.08	0,53 ± 0.06
		1900 g m^{-2}		0.000	1.95 ± 0.01	0.42 ± 0.14
		2600 g m^{-2}		0.092 ± 0.013	2.96 ± 0.20	0.88 ± 0.16
		3200 g m^{-2}		0.123 ± 0.074	1.89 ± 0.03	0.79 ± 0.05
2			Seawater inflow	0.041± 0.005	7.47 ± 0.31	1.28 ± 0.01
			Abalone outflow	1.045± 0.002	8.00 ± 0.11	1.95 ± 0.04
	Gracilaria chilensis	60 L h^{-1}	Seaweed outflow	0.115 ± 0.002	2.14 ± 0.05	0.72 ± 0.02
		80 L h^{-1}		0.120 ± 0.002	3.00 ± 0.20	0.77 ± 0.01
		125 L h^{-1}		0.098 ± 0.012	3.54 ± 0.08	0.76 ± 0.01
		250 L h^{-1}		0.262 ± 0.013	4.83 ± 0.19	1.00 ± 0.03
	Ulva lactuca	60 L h^{-1}	Seaweed outflow	0.083 ± 0.014	1.53 ± 0.07	0.52 ± 0.01
		80 L h^{-1}		0.030 ± 0.003	1.88 ± 0.13	0.63 ± 0.02
		125 L h^{-1}		0.071 ± 0.01	1.96 ± 0.06	0.80 ± 0.01
		250 L h^{-1}		0.141 ± 0.003	3.36 ± 0.09	0.90 ± 0.02
3	*Ulva lactuca*	Autumn	Seawater inflow	0.10 ± 0.07	9.04 ± 0.95	0.78 ± 0.26
			Abalone outflow	1.20 ± 0.77	6.63 ± 0.97	1.09 ± 0.05
			Seaweed outflow	0.54 ± 0.39	2.83 ± 0.45	0.76 ± 0.39
		Winter	Seawater inflow	0.01 ± 0.01	5.04 ± 0.93	0.55 ± 0.14
			Abalone outflow	0.47 ± 0.06	5.47 ± 2.19	0.93 ± 0.32
			Seaweed outflow	0.04 ± 0.01	1.57 ± 0.28	0.64 ± 0.19
		Spring	Seawater inflow	0.38 ± 0.31	4.67 ± 3.12	0.85 ± 0.25
			Abalone outflow	0.75 ± 0.53	5.17 ± 2.81	1.20 ± 0.51
			Seaweed outflow	0.30 ± 0.16	1.95 ± 1.12	0.65 ± 0.20
		Summer	Seawater inflow	0.28 ± 0.09	6.71 ± 5.64	0.75 ± 0.32
			Abalone outflow	0.59 ± 0.12	3.75 ± 1.86	0.82 ± 0.33
			Seaweed outflow	0.16 ± 0.05	2.03 ± 1.39	0.63 ± 0.31

U. lactuca, differences were obtained between the lowest NO_3 uptake efficiency at 250 L h^{-1} (72 ± 3%) when compared to the efficiencies of 125 L h^{-1} (82 ± 2%) and of 80 and 60 L h^{-1} (89 ± 1%) (Tukey, $P < 0.05$).

PO_4 uptake efficiency of *G. chilensis* did not display significant differences at 60, 80, or 125 L h^{-1} (36 ± 7%), however these treatments were significantly higher than the PO_4 uptake efficiency at 250 L h^{-1} (16 ± 9%) (Tukey, $P < 0.05$). In the case of *U. lactuca*, PO_4 uptake efficiency decreased significantly from 60 L h^{-1} (30 ± 8%) to 250 L h^{-1} (14 ± 10%) (Tukey, $P < 0.05$).

At the end of Experiments 1 and 2, *G. chilensis* showed a high degree of epiphytism, principally detected through the abundant presence of brown filamentous algae.

Experiment 3: seasonal nutrient uptake of *U. lactuca*

The uptake efficiency of *U. lactuca* shows a clear seasonal variation for all nutrients studied (ANOVA, F = 778.348, $P < 0.05$), and increased from autumn and reached a peak in the spring before declining again in the summer (Fig. 4). For NH_4, NO_3, and PO_4 the highest uptake efficiencies were recorded in the spring with 100, 64 ± 1, and 47 ± 3%, respectively. The lowest uptake was recorded in the autumn for NH_4 (57 ± 2%) and PO_4 (26 ± 1%), while for NO_3 this was recorded in the summer (49 ± 1%).

Specific growth rate and productivity of *U. lactuca*

U. lactuca showed seasonal variation for SGR (ANOVA, F = 12.470, $P < 0.05$) and productivity

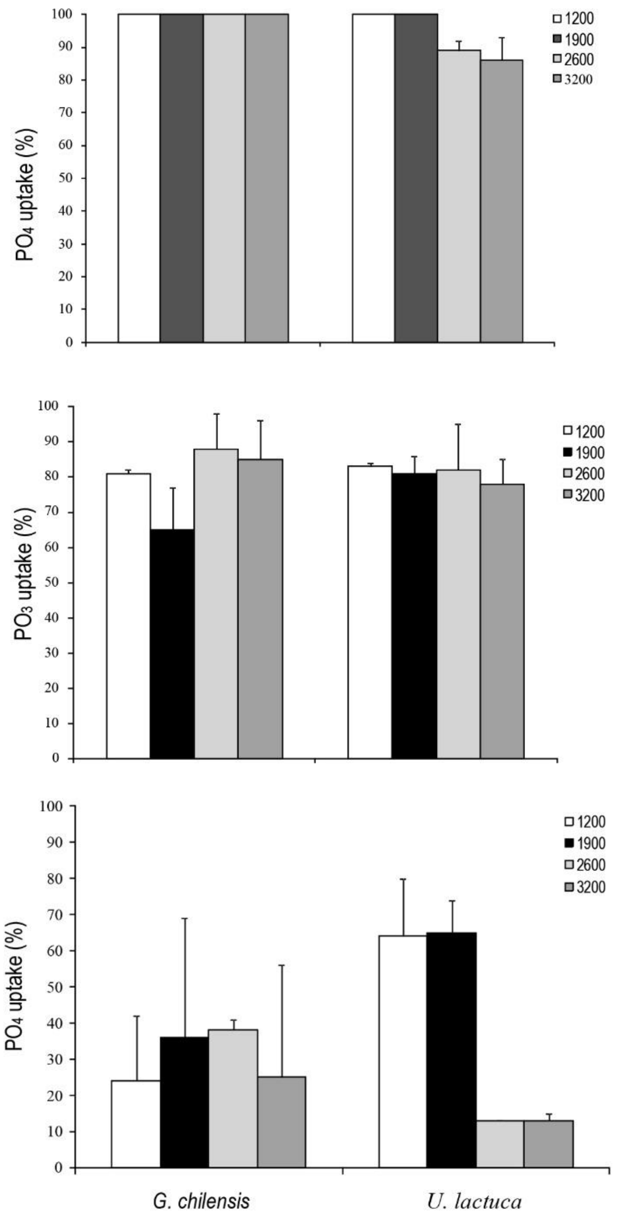

Figure 2. Experiment 1. Nutrient uptake efficiency (NH_4, NO_3, and PO_4) of *Gracilaria chilensis* and *Ulva lactuca*, cultivated at different stocking densities (1200, 1900, 2600 and 3200 g m^{-2}) and receiving discharge water from an abalone culture center. Bars: Mean ± SD.

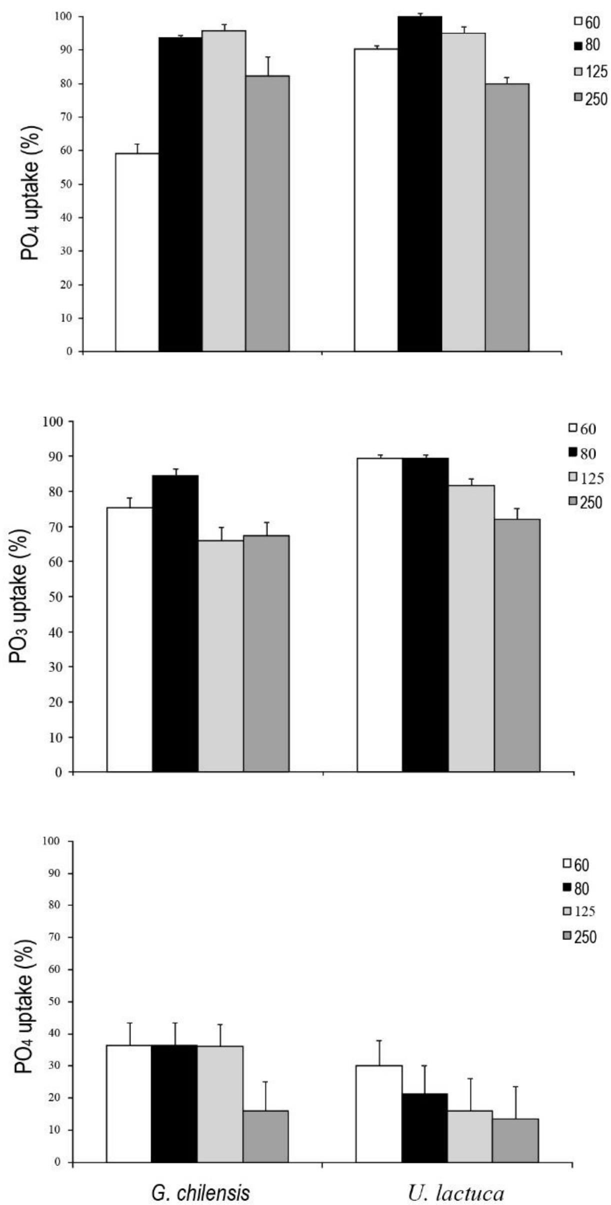

Figure 3. Experiment 2. Nutrient uptake efficiency (NH_4, NO_3 and PO_4) of *Gracilaria chilensis* and *Ulva lactuca*, cultivated at different water exchange rates (60, 80, 125, and 250 L h^{-1}) and receiving discharge water from an abalone culture. Bars: Mean ± SD.

(ANOVA, F = 17.276, $P < 0.05$), with a clear increase from autumn until summer and with ranges between 0.5 ± 0.2 to 2.6 ± 0.2 % d^{-1} and 10 ± 6.1 to 73.6 ± 8.4 g m^{-2} d^{-1} for SGR and productivity, respectively (Fig. 5).

The temperatures registered in seaweed culture tanks were increasing from winter to summer (autumn 15 ± 1; winter 15 ± 2; spring 16 ± 1 and summer 20 ± 1°C).

DISCUSSION

The results show that both *G. chilensis* and *U. lactuca* were efficient in capturing the inorganic nutrients originating from the abalone culture in all of the experimental conditions tested, although there were differences depending on the stocking density and water exchange rate. However, the uptake efficiency of

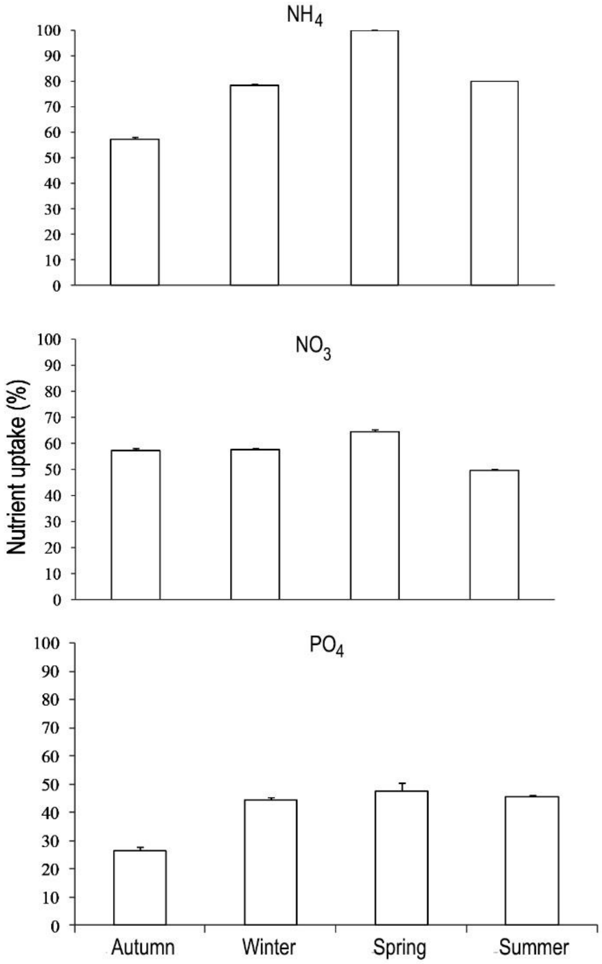

Figure 4. Experiment 3. Seasonal nutrient uptake (NH_4, NO_3 and PO_4) of *Ulva lactuca* cultivated at a stocking density of 1900 g m^{-2}, at a water exchange rate of 125 L h^{-1} and receiving discharge water from an abalone culture. Bars: Mean ± SD.

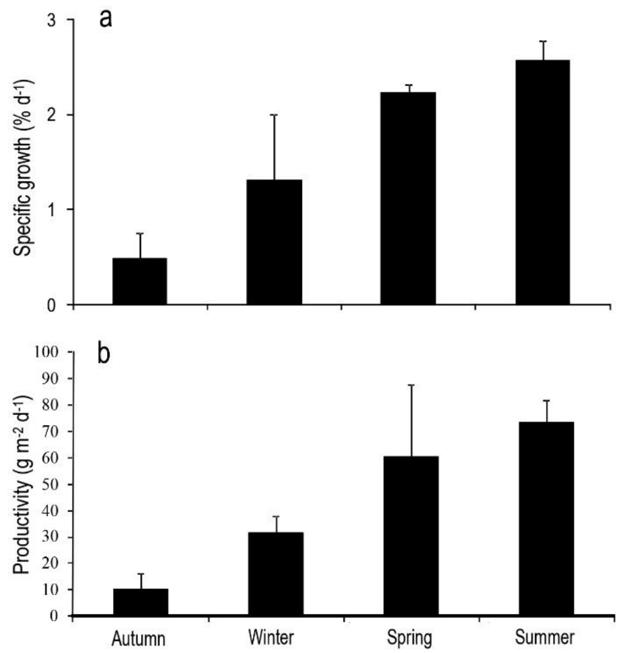

Figure 5. a) Seasonal specific growth rate (% d^{-1}), and b) productivity (g m^{-2} d^{-1}) of *Ulva lactuca* cultivated at a stocking density of 1900 g m^{-2}, water exchange rate of 125 L h^{-1}, and receiving discharge water from an abalone culture. Bars: Mean ± SD.

NH_4, NO_3, and PO_4 registered for both species was greater than the contribution of these nutrients given by the abalone culture, translating into a guaranteed total remediation of treated wastewater. Of the inorganic nutrient sources evaluated in this study, NH_4 was the most absorbed by *U. lactuca* and *G. chilensis* for both the stocking density and water exchange rate experiments. In this same line, the ability of algae to capture NO_3 and NH_4 has been well described, with various studies reporting the high affinity of both *Gracilaria* (Jones *et al*., 2001; Abreu *et al*., 2011) and *Ulva* (Harlin *et al*., 1978; Thomas & Harrison, 1987) in efficiently capturing NH_4.

The importance of seaweed stock density for the productivity and nutrient uptake efficiency in a biofiltration system has been widely documented (Neori *et al*., 1991; Chopin *et al*., 2001; Zhou *et al*., 2006). In Experiment 1, *G. chilensis* demonstrated a high uptake efficiency for the three sources of inorganic nutrients, which was clearly evidenced by the total removal of NH_4 in all stocking densities tested. These results confirm those reported for diverse species such as *Gracilaria vermiculophylla* (Abreu *et al*., 2011), *Gracilaria bursa pastoris* (Matos *et al*., 2006) and *U. lactuca* (Cohen & Neori, 1991), where stocking density has resulted in being inversely proportional to nutrient uptake. Furthermore, it has been reported that a high stocking density affects an adequate access to light, producing shading and limiting photosynthesis which ultimately diminished the productivity of the algae and thus the demand for nutrients (Matos *et al*., 2006). Given these results, it is possible to determine that the maximum density permitted to guarantee the greatest uptake of nutrients in *U. lactuca* is 1900 g m^{-2}, similar to the stocking density described by Robertson-Andersson *et al.* (2008) for this same species. This same stocking density could be recommended for *G. chilensis*, despite that uptake efficiency is maintained even at 2600 and 3200 g m^{-2}. However, a lower density would permit a better operating control of the culture. This stocking density is lower than the 3000 g m^{-2}, reported by Abreu *et al*. (2011) for *G. vermiculophyta* and the 7000 g m^{-2} recommended by Chow *et al.* (2001)

when using *G. chilensis* for the treatment of wastewater originating from fish and oysters.

It is expected that the greatest uptake efficiency would be reached with the lowest water exchange rate (Chopin *et al.*, 2001). This situation was clearly observed for *U. lactuca* where lower uptake efficiency was measured for all three nutrients at the high WER of 250 L h^{-1}, a situation which was repeated for *G. chilensis* with the uptake of PO_4. However, a contrary situation was observed for *G. chilensis* where a lower uptake efficiency of NH_4 and NO_3 was observed at 60 L h^{-1}. Despite that the cultures were maintained with constant aeration, these results could be associated with a deficiency of inorganic carbon (IC), which has already been mentioned as the first element that diminishes in a tank cultivation system (Craigie & Shacklock, 1995) and that directly affecting the capacity for photosynthesis. On the other hand, high epiphyte load detected on *G. chilensis* at the end of experiments too could explain the reduction of its uptake efficiency.

On the other hand, temperature has also been mentioned as a factor which limits uptake efficiency, especially when a low water exchange rate is maintained in systems exposed to varying local conditions, as were used in this study (Abreu *et al.*, 2011). However, during this study, no temperature increase was detected that exceeded the tolerance limits of each species, but this factor should be given special consideration when performing cultivations in the north of Chile. For this, a water exchange rate of 125 L h^{-1} is recommended for both species, which will effectively diminish nutrient concentration, cleaning a greater volume of wastewater while ensuring adequate conditions for algae cultivation.

At the end of Experiments 1 and 2, *G. chilensis* showed an important level of epiphytism, principally being affected by filamentous algae. Concerning this subject, several descriptions have been made mentioning epiphytism for *Gracilaria*, both for massive sea cultivations and in tanks (Oliveira *et al.*, 2000), which is one of the principal problems for the commercial aquaculture of this species. Some authors have noted severe problems of epiphytes in algae used in biofiltration systems (Robertson-Andersson *et al.*, 2008), affecting growth and, in some cases, producing a total loss of biomass. Instead, *U. lactuca* remained free of epiphytes and was thus chosen for the seasonal experiments.

During Experiment 3 it was possible to observe that uptake efficiency was clearly seasonal for the three nutrients evaluated, increasing in autumn and reaching a maximum efficiency in spring before diminishing in summer. This result allows a total removal of the NH_4 produced by the abalone culture in spring, while in autumn, winter and summer, only an uptake efficiency of 57 ± 2%, 79 ± 2% and 80 ± 2% respectively, is achieved. On the other hand, although the uptake efficiency for NO_3 and PO_4 was less than NH_4 in all seasons, it allowed a total removal of the nutrients produced by the abalone during the entire year. These results show high uptake efficiency in eliminating NH_4, NO_3, and PO_4 waste of the abalone *H. rufescens* cultivated on the coast of Chile. This is in agreement with various experiences in different latitudes which mentioned *U. lactuca* as one of the best candidates for use in a biofiltration system with abalones and other species (Cohen & Neori, 1991; Neori *et al*., 1996, 1998, 2000; Schuenhoff *et al*., 2002; Robertson-Andersson *et al*., 2008; Bolton *et al*., 2009; Da Silva *et al*., 2009).

Although the principal objective of this study was to determine the nutrient uptake efficiency of two algae from the Chilean coast, growth rate and seasonal productivity were also evaluated for *U. lactuca*, given the importance that the algae could have for the same abalone culture as a food source for adult specimens (Mai *et al*., 1994; Shpigel *et al.*, 1996; Corazani & Illanes, 1998; Shpigel *et al.*, 1999; Troell *et al.*, 2006) or as a source of propagules for the settlement and feeding of abalone larvae and postlarvae (Muñoz *et al*., 2012), as well as for other increasingly used purposes, such as a healthy food option for humans (Higashi-Okai *et al*., 1999; Yamori *et al*., 2001; Aceves *et al*., 2005).

The seasonal increase in growth rate and productivity of *U. lactuca* from autumn to summer has a close relationship with the seasonal uptake efficiency of NH_4, NO_3, and PO_4, as more nutrients are being captured during greater growth. This seasonal variation in biomass gain has been found among various species of algae used in integrated cultivation systems (Matos *et al*., 2006; Robertson-Andersson *et al*., 2008). As it has been reported several times, this situation appears to be predominately dependent on the seasonal variation of temperature and light (Neori *et al*., 1998), two recognized factors controlling the growth of algae (Bulboa & Macchiavello, 2001).

For the results registered in this study, we conclude that there is a sufficient basis for demonstrating the high possibility for changing the traditional, monoculture system of abalone in Chile to one of a sustainable IMTA system, including *U. lactuca* as a biofiltration unit to reach positive environmental externalities.

In this context, to reduce the NH_4, NO_3, and PO_4 output of an abalone production center with an approximate outflow of 100000 L h^{-1}, a total biomass of 760 kg of *U. lactuca* distributed in tanks at a stocking density of 1900 g m^{-2} and with a water exchange rate of

125 L h^{-1} would be needed. These installations would occupy an area of approximately 400 m^2.

Our results confirm that *U. lactuca* should be considered a serious candidate in the implementation of wastewater treatment plans from abalone cultures in Chile, as it presents important advantages such as high uptake efficiency, wide distribution and abundance, resistance to environmental variations, easy culture management, and scarce epiphytism.

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