

## Full Length Research Paper

# Bio-physiological response of biofilter algal candidate *Ulva* sp. to different nitrogen forms and fluxes

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The present study was carried out to study the long term effect of different nitrogen forms and concentrations (14.3, 28.6 and 57.1  $\mu\text{M}$  N-NH<sub>4</sub> and 214, 2360 and 3700  $\mu\text{M}$  N-NO<sub>3</sub>) on the photosynthesis and relative growth rate (RGR) of *Ulva* sp. for five weeks. Nutrient-enriched seawater was supplied at an exchange rate of between 12.6 and 14.4 volumes per day. Relative growth rate was determined weekly. Photosynthetic oxygen evolution response was measured using 20 - 25 mg fresh weight seaweed incubated in a DW3 measuring chamber for 56 min under different irradiances with light and dark periods alternating every seven minutes. Photosynthetic light-response curves were drawn using 15 photosynthetic oxygen evolution readings normalized to dry weight. Experimental data were fitted with a Haldane model to calculate photosynthetic rate ( $P_{max}$ ), saturation irradiance ( $I_s$ ) and compensation irradiance ( $I_c$ ). At the experiment termination, the RGR following nitrate addition were higher, but not significantly, than those with the ammonium supplied. The highest RGR was determined at the medium nitrate concentration. The photosynthetic activity of *Ulva* sp. showed a positive relationship with nitrogen concentrations from both nitrogen forms. The highest significant  $P_{max}$  was found at the highest nitrate concentration. The lowest ammonium concentration corresponded to the lowest significant  $P_{max}$  value. No significant differences were found for  $I_s$  and  $I_c$  irrespective of treatment, although, in general, ammonium treatments yielded higher  $I_s$  values than nitrate treatments. The discrepancy between the growth rate results and photosynthetic oxygen evolution is discussed in light of the reproduction activity and temperature effect.

**Key words:** *Ulva* sp., photosynthetic oxygen evolution, relative growth rate, nitrogen forms, nutrients uptake.

## INTRODUCTION

As the aquaculture industry is developing worldwide, a huge amount of effluent is released into the environment. To limit the release of farm effluent into the environment and promote the sustainable development of aquaculture industry, waste water treatment needs to be optimized. Macroalgae have been successfully integrated into various-scale mariculture systems including flow through systems (FTS), semi-recirculating and recirculating systems

(Pagand et al., 2000; Schuenhoff et al., 2003). The effluents of different aquaculture production systems, such as FTS and recirculating aquaculture systems (RAS) contain various forms and concentrations of nitrogen, depending on how the systems are managed. The effluent of FTS is characterized by high flow rates and low ammonia and phosphorus concentrations whereas RAS effluent is characterized by low flow rates and high nitrate and phosphorus

concentrations which may reach concentrations 10 to 100 fold those of conventional systems (Neori, 1996; Pagand et al., 2000; Blancheton et al., 2007). To optimize integrated *Ulva* - fish culture systems, growth rates and photosynthetic activity need to be evaluated as a function of nitrogen forms and concentrations.

Algal uptake of nitrogen depends on numerous factors including physical factors (light, temperature), environmental nutrient concentration, intracellular nutrient concentrations and biological factors (metabolism, age, etc.). Studies on nitrogen form preferences are conflicting. Several studies on nitrogen enrichment of *Ulva* sp. show that ammonium ( $\text{NH}_4^+$ ) is physiologically preferred over nitrate ( $\text{NO}_3^-$ ) given that less energy is required for its assimilation into the algal biomass (Thomas and Harrison, 1985; Fujita et al., 1989). Macroalgae also show higher growth rates with  $\text{NH}_4^+$  than with  $\text{NO}_3^-$  (DeBoer et al., 1978; Lapointe and Ryther, 1979). Ale et al. (2011) reported that ammonium is favourably assimilated by *Ulva lactuca*; giving rise to a higher RGR and biomass yield and the uptake rate of ammonium is higher than that of nitrate. However, at the end of a 10-day culture period, the biomass yield of *U. lactuca* with the nitrate treatment was higher than that with the ammonium treatment. Moreover, Luo et al. (2012) showed that after 180 min of incubation of *Ulva prolifera* and *Ulva linza* in media enriched with either nitrate or ammonium, the  $V_{max}$  (maximum uptake rate) for nitrate was higher than that for ammonium in both species. The  $V_{max}/K_s$  ( $K_s$  is the half-saturation constant for nutrient uptake) ratio was very similar for the two nitrogen forms in both *Ulva* species, which may indicate that over a longer period, both species may exhibit higher affinity for nitrate than ammonium. The presence of  $\text{NH}_4^+$  has been shown to reduce the uptake of  $\text{NO}_3^-$  in macroalgae (Thomas and Harrison, 1987) at concentrations  $>2 \mu\text{M}$   $\text{NH}_4^+$  (D'Elia and DeBoer, 1978; Neori, 1996). However, Ale et al. (2011) showed that, in the presence of  $\text{NH}_4\text{NO}_3$ , *U. lactuca* showed a relatively higher uptake rate of  $\text{NO}_3^-$  as compared to when exposed to  $\text{NaNO}_3$  alone.

Nitrate is usually more abundant than ammonium in coastal and estuarine water and it makes up more than 80% of dissolved inorganic nitrogen in effluents of recirculating systems containing nitrifying filters (Neori, 1996; Pagand et al., 2000). Furthermore, macroalgal proliferation has been observed in estuaries that receive nitrate-rich wastewater (Cole et al., 2005). Nitrogen accumulation studies show that nitrate is less toxic at high concentrations than ammonium (Andersson, 1942) and that it is taken up at similar rates to ammonium (Pagand et al., 2000). Similar growth rates with either  $\text{NH}_4\text{-N}$  or  $\text{NO}_3\text{-N}$  have been reported for different seaweed species

(Navarro-Angulo and Robledo, 1999; Carmona et al., 2006). Additionally, the growth of *Ulva fasciata* has been found to increase with increasing  $\text{NO}_3^-$  concentrations under high lighting conditions (Lapointe and Tenore, 1981).

Most studies conducted to date have focused on the uptake rate of N because N availability often limits the growth of macroalgae (Hanisak, 1983). Moreover, these studies were conducted in the form of short-term (from hours to days) batch experiments and investigated low concentration ranges, 5 - 200  $\mu\text{M}$  (Fujita et al., 1989; Naldi and Wheeler, 1999; 2002; Ale et al., 2011; Luo et al., 2012), which is different from the conditions of seaweeds in bio-filters, fed high concentration of nutrients in a continuous flow for long periods.

Therefore, the purpose of this study was to monitor the growth and photosynthetic activity of *Ulva* sp. in the long term with different nitrogen forms and concentrations, to gain a better understanding of the effect of nitrogen form and concentration on the filtration performance of *Ulva* during the fish production cycle. This information will be useful for designing algal ponds for the purification of aquaculture waste water and will help us predict the filtration performance of *Ulva* throughout the fish production cycle.

## MATERIALS AND METHODS

### Plant collection and acclimation

Vegetative fragments of *Ulva* sp. were collected from in a natural pond close to the IFREMER Palavas station during May and June, 2009. They were rinsed with filtered sea water and gently cleaned to remove epibiota and sediments, and then cut into small 6  $\text{cm}^2$  pieces, to keep them suspended and moving within the experimental bottles. Before the experiments, the plants were maintained in the laboratory for a preconditioning period of 2 weeks at  $25 \pm 1^\circ\text{C}$  subjected to a 12:12 hours L:D cycle at  $273 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ; the same temperature and light conditions were maintained during all subsequent experiments. Irradiance was provided by four cool white fluorescent tubes (OSRAM Trade Brand 150 cm, 58 W, Germany). Light in the photosynthetically active range (PAR) was measured with a spherical sensor connected to a LiCor-1400 Data Logger radiometer (Li-Cor Inc, Lincoln, Nebraska, USA). Further details of selected temperature and light intensity levels were selected as given in the discussion. During the preconditioning period, the plants were stocked at a density of 5 g (FW)  $\text{L}^{-1}$ . Filtered seawater was enriched with 1 mM of either  $\text{NO}_3\text{-N}$  ( $\text{KNO}_3$ ) or  $\text{NH}_4\text{-N}$  ( $\text{NH}_4\text{Cl}$ ) and  $\text{PO}_3\text{-P}$  ( $\text{KH}_2\text{PO}_4$ ) at a N:P ratio of 20 (w:w) and renewed every 2-3 days. Aeration was provided continuously.

### Growth experiment

For five weeks, twelve 1-L flasks received a continuous flow of

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Figure 1. The experimental system.

seawater enriched with ammonium or nitrate at concentrations of 14.3, 28.6, 57.1  $\mu\text{M}$   $\text{NH}_4\text{Cl}$  (T1; T2 and T3) or 0.214, 2.36, 3.7  $\text{mM}$   $\text{KNO}_3$  (T4; T5 and T6), respectively (two flasks for each nitrogen concentration) from storage tanks with a 40 L capacity. Phosphate ( $\text{KH}_2\text{PO}_4$ ) was added to all treatments at a N:P ratio of 20 (w:w). Nutrient-enriched seawater was supplied at flow rates ranging between 12.6-14.4 volumes per day. The flow rates were adjusted manually every day. These nutrient concentrations were selected to cover the concentration range of nutrients reported in the literature for two fish production systems FTS and RAS. At the beginning of the experiment, the flasks were stocked algae at a density of 5 g FW/ flask (Figure 1).

### Growth rate determination

The fresh weight of the plants in each flask was determined weekly and the initial density was readjusted. Relative growth rates (RGR %  $\text{d}^{-1}$ ) were estimated using the following formula:  $\text{RGR} = \ln(W_t/W_0) \times \Delta t^{-1} \times 100$ , where  $W_0$  is the initial biomass and  $W_t$  is the biomass on day  $t$  and  $\Delta t$  the time period (Evans, 1972).

### Photosynthetic oxygen evolution experiment

Six flasks were used for the determination of photosynthetic oxygen evolution in response to the different nitrogen forms and concentrations. The algae were kept under similar culture conditions throughout the growth experiment, which used the same experimental system. The photosynthetic oxygen evolution rate was measured daily for 5 weeks. Measurements with each nitrogen concentration were repeated 25 times.

### Measurement of photosynthetic oxygen evolution

Samples (20 - 25 mg fresh weight or FW) were taken from the algae at each nitrogen concentration using a sharp razor blade. Photosynthetic  $\text{O}_2$  evolution was measured with a Clark-type oxygen electrode in a DW3 measuring chamber (Hansatech Instruments, Norfolk, UK) at  $25^\circ\text{C}$  and at different irradiances (from 300 to 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). The algae samples were incubated in the measuring chamber containing 13 ml of filtrated natural seawater.  $\text{NaHCO}_3$  stock solution (200  $\text{mM}$ ) was used at a

rate of 0.8 ml per 13 ml seawater to provide non-limiting dissolved inorganic carbon during the incubation period. Each measuring trial consisted of a 56 minute sequence during which the algae were exposed to 7 min of alternating light and darkness. The photosynthesis measurements were conducted between 10 am and 6 pm.

Photosynthesis measurements were started after the first hour of illumination to avoid induction phenomena (Henley et al., 1991). The treatments were alternately sampled through the experimental period. The small pieces of *Ulva* were incubated in filtered seawater, at 273  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  and  $25^\circ\text{C}$  for at least 1 h to minimize the effect of any potential cutting damage of cells (wound respiration) on photosynthetic measurements (Zou, 2005).

Photosynthetic oxygen evolution rates were normalized to dry weight (DW). Data obtained from measurement of the photosynthetic rates used to draw net photosynthesis versus irradiance ( $P-I$ ) curves were the means of 15 out of 25 readings. To evaluate photosynthetic activity ( $P$ ) taking photoinhibition into account, experimental data were fitted with a Haldane model (Equation 1), as modified by Papacek et al. (2010):

$$P = P_{\max} \times \frac{I}{K_s + I + I^2/K_i} - R_d \quad (1)$$

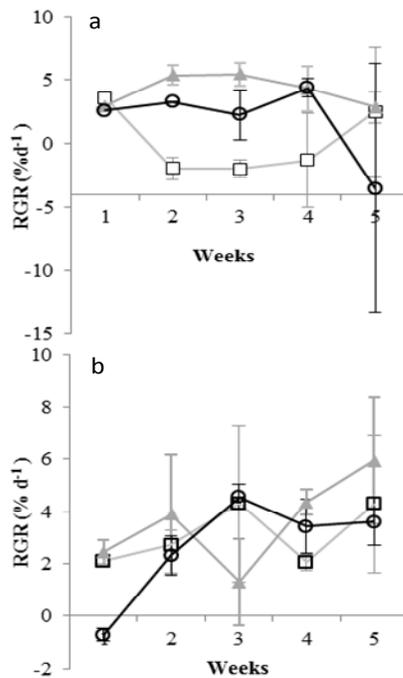
where  $P_{\max}$  is the maximal photosynthetic capacity ( $\mu\text{mol O}_2 \text{gDW}^{-1} \text{h}^{-1}$ ),  $K_i$  is the inhibition constant ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ),  $K_s$  is the half-saturation constant ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and  $R_d$  is the dark respiration expressed as oxygen consumption ( $\mu\text{mol O}_2 \text{gDW}^{-1} \text{h}^{-1}$ ). Saturation irradiance ( $I_s$ ) and compensation irradiance ( $I_c$ ) were calculated according to Equations 2 and 3.

$$I_c = \frac{P_{\max} - R_d + \sqrt{(R_d - P_{\max})^2 - (2R_d \sqrt{K_s K_i})^2}}{2R_d/K_i} \quad (2)$$

$$I_s = \sqrt{K_s K_i} \quad (3)$$

### Statistical analysis

The Haldane model was fitted to data using Matlab (version 6.00) software. The data were analyzed statistically using one way ANOVA (Microsoft office Excel 2007) and expressed as the means  $\pm$  SE. Differences among treatments were tested for significance



**Figure 2.** Mean relative growth rate (% per day) of *Ulva* sp. with (a) different ammonium levels, squares (T<sub>1</sub>), triangles (T<sub>2</sub>) and circles (T<sub>3</sub>), corresponding to 14.3, 28.6, 57.1  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , respectively and (b) nitrate levels, squares (T<sub>4</sub>), triangles (T<sub>5</sub>) and circles (T<sub>6</sub>), corresponding to 0.214, 2.36, 3.7 mM  $\text{KNO}_3$ , respectively. Error bars represent SE values (n=2).

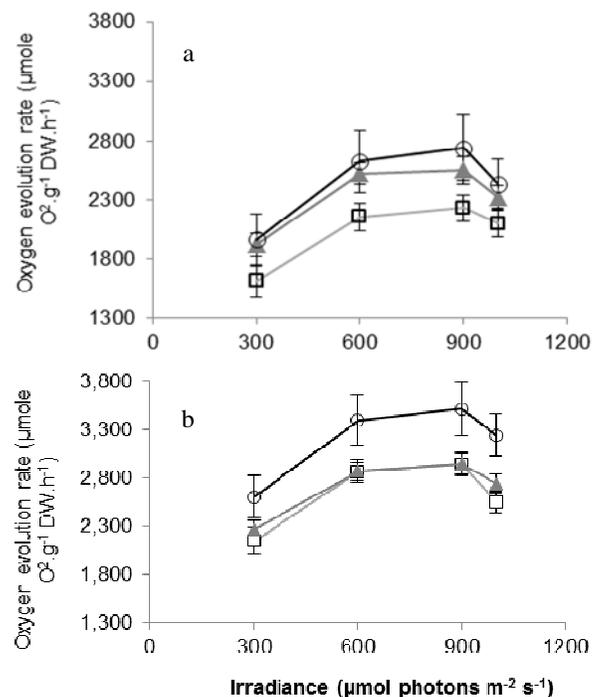
with the *t*-test with the significance level set at 0.05.

## RESULTS

### Growth rate

The relative growth rate (RGR) of the algae is shown in Figure 2. After one week, the RGR with the lowest ammonium concentration (T<sub>1</sub>) was the highest and differed significantly ( $P < 0.05$ ) from the RGR of the algae receiving nitrate treatment (T<sub>4, 5</sub> and 6), but not significantly from those with the other concentrations of ammonium (T<sub>2</sub> and 3). After two weeks, it became the significantly lowest RGR ( $P < 0.05$ ).

At the end of the experiment, the RGR with the three nitrate treatments were higher, but not significantly, than those with ammonium treatments. The highest RGR was observed with the medium nitrate concentration (T<sub>5</sub>) (Figure 2). A positive trend over time was observed for RGR with the nitrate treatments, while no particular trend was noticed for RGR in the ammonium treatments. Generally,



**Figure 3.** Photosynthetic oxygen evolution with (a) different ammonium levels, (a) different ammonium levels, squares (T<sub>1</sub>), triangles (T<sub>2</sub>), and circles (T<sub>3</sub>), corresponding to 14.3, 28.6, 57.1  $\mu\text{M}$   $\text{NH}_4\text{Cl}$ , respectively and (b) nitrate levels, squares (T<sub>4</sub>), triangles (T<sub>5</sub>) and circles (T<sub>6</sub>), corresponding to 0.214, 2.36, 3.7 mM  $\text{KNO}_3$ , respectively. Error bars represent SE values (n=15).

algae receiving nitrate, particularly medium and high concentrations, showed a biweekly reproduction rhythm.

### Photosynthetic activity

The photosynthetic activity of *Ulva* showed a positive relationship with nitrogen concentrations for both nitrogen forms (Figure 3 and Table 1). All nitrate treatments yielded higher  $P_{max}$  values than the ammonium treatments. The highest photosynthetic rate ( $P_{max}$ ) was recorded in response to the highest nitrate addition, followed by that at the medium nitrate concentration. These values were significantly ( $P < 0.05$ ) higher than any other N treatments. The lowest ammonium concentration corresponded to the lowest  $P_{max}$  values. All the nitrate concentrations resulted in higher photosynthesis rates for a given irradiance level (Table 1).

In general, the ammonium treatments yielded higher saturation irradiance  $I_s$  values than the nitrate treatments. The lowest value of  $P_{max}$  ( $595 \pm 35 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) yielded at the lowest ammonium concentration (T<sub>1</sub>). No significant differences were detected among the treatments, the values ranged between  $595 \pm 35$  to  $658 \pm 31 \mu\text{mol}$

**Table 1.** The photosynthetic parameters values (mean  $\pm$  SE) under different nitrogen sources and concentrations. Significant differences among groups as identified by pairwise contrasts are indicated by different letters. (n=15).

N source	$I_c$	$I_s$	$P_{max}$
NH <sub>4</sub> _0.2 mg N/L	48 $\pm$ 6	595 $\pm$ 35	2532 $\pm$ 112.66c
NH <sub>4</sub> _0.4 mg N/L	45 $\pm$ 5	619 $\pm$ 36	2625 $\pm$ 97.83c
NH <sub>4</sub> _0.8 mg N/L	51 $\pm$ 6	658 $\pm$ 31	2745 $\pm$ 262.33c
NO <sub>3</sub> _3 mg N/L	47 $\pm$ 6	615 $\pm$ 47	3013 $\pm$ 250.41c
NO <sub>3</sub> _33 mg N/L	47 $\pm$ 5	606 $\pm$ 22	3034 $\pm$ 160.18b
NO <sub>3</sub> _53 mg N/L	57 $\pm$ 3	598 $\pm$ 26	3644 $\pm$ 234.27a

Within each column, different letter indicates significant differences at  $P < 0.05$ .

photon  $m^{-2} s^{-1}$  (Table 1).

No significant differences were found for predicted compensation point values ( $I_c$ ) between the different nitrogen forms and concentrations. The lowest predicted  $I_c$  value was found at the medium ammonium concentration (T<sub>2</sub>), that is,  $45 \pm 5 \mu\text{mol photon } m^{-2} s^{-1}$ , while the highest predicted  $I_c$  value was found with the highest nitrate concentration (T<sub>6</sub>), that is,  $57 \pm 3 \mu\text{mol photon } m^{-2} s^{-1}$  (Table 1).

## DISCUSSION

Vandermeulen and Gordin (1990) reported that increasing flow rates in culture tanks up to 8 volumes  $day^{-1}$  significantly increased growth rates of *Ulva*. In this study, *Ulva* sp. were maintained in a continuous flow rate of nutrients of 12-14 volumes  $day^{-1}$ , which is comparable to the flow rates reported in several studies, that is, 12-18.8 volumes  $days^{-1}$  (Fujita et al., 1989; Neori et al., 1991; Figueroa et al., 2009). The maximum growth rate for *Ulva* sp. presented here (8.37 %  $d^{-1}$  at the end of the growth experiment with the medium nitrate concentration) is comparable to the maximum growth rates of 7.86%  $d^{-1}$  for *Ulva* sp. reported by Rosenberg and Ramus (1981), = 7%  $d^{-1}$  for *Ulva pertusa* (de-Casabianca et al., 2002) and the growth rate obtained by Floreto et al. (1993) at a similar density (of 5g FW/L) of *U. pertusa* using Provasoli medium.

The higher growth rates reported in other studies for *Ulva rigida* (Lavery and McComb, 1991), *U. lactuca* (Neori et al., 1991) may be partially attributed to the use of lower stocking density (1.67 - 3.6 g/L) (Neori et al., 1991; Figueroa et al., 2009) as compared to that of the present study (5 g FW/L). Some studies (Lapointe and Tenore, 1981; Mata and Santos, 2003) have reported that the optimal stocking density is about 0.8 - 1.3 kg  $m^{-2}$  (corresponding to 1.4-2.4 g/L FW). Some authors have reported that increasing stocking density decreases the growth rate and yield of *Ulva* (DeBusk et al., 1986; Neori et al., 1991) and other algal species (Neish et al., 1977;

Lapointe and Ryther, 1978). However, these studies were carried out in the field and the algae received fish effluent. Fujita et al. (1989) found that growth rates of *Ulva rigida* were higher in outdoor cultures (10%  $d^{-1}$ ) than in indoor cultures (4%  $d^{-1}$ ). Additionally, Neori et al. (1991) found that fish pond effluents produced higher yields (by up to 38%) than pulse nutrient addition and Floreto et al. (1993) reported that a high nutrient turnover in natural conditions may explain higher algal growth rates as compared to indoor culture environments.

Israel et al. (1995) reported that the optimal mean temperature for growth of *U. lactuca* is about 20°C, but higher temperatures may favour growth during spring. Many studies have reported that the optimal temperature for growth of *Ulva* sp. is 23 - 24°C (Riccardi and Solidoro, 1996). Therefore, the applied temperature (25  $\pm$  1°C) in the present study may be considered relatively high, particularly for the *Ulva* species collected on French shores. It could explain, to some extent, the reduced growth rate observed during this work. de-Casabianca et al. (2002) showed that growth in *U. rigida* was limited at temperatures outside the range 7 - 25°C and that temperatures higher than the optimal values (from 12 to 23°C) reduce the growth of *U. rigida* collected from the same site as that used for the present study. However, Mohsen et al. (1973a) found that at 25°C, maximum amino acids and sugar contents were reached in *U. fasciata*, from Alexandria in Egypt.

This relatively high temperature was applied to control reproduction activity in the present study. Nordby (1977) reported that the optimal temperature for reproduction in species of *Ulva mutabilis* is 21 - 22°C and that temperatures higher than 24°C resulted in a drop in sporulation. Furthermore, Nordby (1977) showed that maximum sporulation was achieved at light intensities between 22.8 - 152  $\mu\text{mol } m^{-2} s^{-1}$ . Therefore, to inhibit reproduction in the present study, the temperature was maintained at 25  $\pm$  1°C and irradiance at a high level (273  $\mu\text{mol photons } m^{-2} s^{-1}$ ) since gamete formation is usually enhanced when temperatures are lowered to less than

20°C (Mohsen et al., 1973a). These conditions, however, did not inhibit reproduction, particularly with the nitrate treatments. In addition, the pH of the water in the experimental bottles ranged between 8 - 8.76, which is almost the optimal pH range for *Ulva mutabilis* sporulation according to Nordby (1977).

A strong positive correlation between light intensity and growth rate was noticed up to 35  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  while above 42  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , bleaching was observed in thalli (Mohsen et al., 1973b). However, according to Ramus and Venable (1987), optimal irradiance values are between 400 and 500  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for *U. rigida* growth, and between 200-500  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for *Ulva curvata* (Coutinho and Zingmar, 1993).

The differences in growth rates of *Ulva* sp. among published studies may also be attributed to a combination of different culture conditions, that is, light quality (Steffensen, 1976), types of lamps, intensity (Mohsen et al., 1973b; Bjornsater and Wheeler, 1990) and culture management (Steffensen, 1976), or use of culture media enriched with macro and micro-nutrients as well as vitamins (Floreto et al., 1993), and lower stocking densities 0.8-2.0 g FW/L (Bjornsater and Wheeler, 1990; Floreto et al., 1993).

Floreto et al. (1993) showed that form of nitrogen significantly affected growth of *U. pertusa* during the 6 first days of culture, with factors such as temperature becoming dominant after that. According to that, during the first week of our experiment, the lowest ammonium concentration, at a light intensity of 273  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and a temperature of 25°C, was found to be the optimal for growth, resulted significantly higher RGR than all the nitrate treatments. However, the highest nitrate concentration under the same conditions was the more effective triggering factor for reproduction, which might have masked the real RGR under this treatment.

In a study on *U. lactuca* RGR response according to nitrogen form, Ale et al. (2011) noticed a decrease in RGR after 5 - 6 days of culture in media enriched with ammonium or nitrate, respectively. Similarly, Bjornsater and Wheeler (1990) noticed that after 5 - 7 days of maintaining *Ulva fenestrata* under 200  $\mu\text{M NO}_3\text{-N}$  and 12  $\mu\text{M PO}_4\text{-P}$ , the growth rate decreased from 30 to about 10 %  $\text{d}^{-1}$ , and then fluctuated between 20 and less than 10%  $\text{d}^{-1}$  during the remaining experimental period. Similar fluctuations were also recorded in the present study which indicates that it may be a normal phenomenon in *Ulva*. Moreover, de-Casabianca et al. (2002) found that high fluctuations (from 0 to 8%  $\text{d}^{-1}$ ) could be observed in RGR of *U. rigida* in nature.

*Ulva* spp. reproduction may be triggered by many factors (change in nutrient concentrations or handling of algae) and results in loss of part of the yield (Steffensen, 1976; Neori et al., 1991). Mohsen (1974a, b) reported that a rich supply of nitrogen promotes asexual reproduction in *U. fasciata* while sexual reproduction is enhanced by

nitrogen depletion. This explains the difficulties encountered in determining the RGR in natural *Ulva* in which sporulation may easily be induced by changes in any of the culture conditions (temperature, light, nutrients, culture media, etc) (Mohsen et al., 1973a, b; Thiadens and Zeuthen, 1976) or by weighing procedures (Bjornsater and Wheeler, 1990). The authors also reported that N tissue contents may have an effect on sporulation activity. Therefore, to avoid reproduction interference, sterile clones of *Ulva* were used (DeBusk et al., 1986) or short-term, indoor experiments never exceeding 14 days (Fujita et al., 1989; Naldi and Wheeler, 2002) were conducted.

The results of this study indicate that moving *Ulva* into higher nitrate concentrations (from 1 mM, during the preconditioning period to 3.8 mM) triggered their reproduction faster than moving them to lower concentrations, and this may have masked their real RGR in that treatment. Nitrate nitrogen induced a fortnight reproduction pattern, while no particular pattern was noticed with the ammonium treatments. Many *Ulva* species have been found to release swarms in a 14-15 day cycle (Rhyne, 1973), which explains RGR fluctuation, particularly with the nitrate treatments.

The highest  $P_{max}$  was found for algae maintained under the highest nitrate concentration (3.8 mM), while the highest RGR achieved during this experiment was with the medium concentration of nitrate (2.36 mM). This apparent discrepancy could be explained by the intense reproductive activity observed at the highest nitrate concentration, which might have overridden the real growth rate with this treatment.

The irradiance-saturated photosynthetic rate does not necessarily reflect growth rate since it is related to electron flow between PSII and PSI (Dubinsky et al., 1986). However, a higher  $P_{max}$  value results from an increase in RuBPCase activity, which means that more inorganic nitrogen could be used for this purpose (Coutinho and Zingmark, 1993). In addition,  $P_{max}$  is dependent on light-independent reactions which are largely controlled enzymatically (Bannister, 1974), which may lead to higher nutrients metabolism rates or at least metabolites. Photosynthesis has been reported to be correlated with growth in *U. lactuca* (Israel et al., 1995) and in *Gracilaria tikvahiae* (Lapointe and Duke 1984) where carbon fixed during photosynthesis is rapidly converted into organic matter with minimum accumulation. Figueroa et al. (2009) reported that high nitrogen supplies resulted in higher electron transport rates, which was reflected in higher photosynthetic activity in *U. lactuca*. Geider et al. (1993) also found that nitrate nitrogen supply affects photosynthetic performance in *Phaeodactylum tricornutum* (Bacillariophyceae). Moreover, in the present study, the lower  $P_{max}$  and  $I_c$  values observed with ammonium treatments indicate lower photosynthesis efficiency than the higher  $P_{max}$  and  $I_c$  values observed at the highest nitrate concentration, as evidenced by the slopes of the curves.

The light intensity of 320 - 360  $\mu\text{mol m}^{-2}\text{s}^{-1}$  was found to be saturating for photosynthesis of *Ulva fenestrata* (Bjornstater and Wheeler, 1990) and 300 - 370  $\mu\text{mol m}^{-2}\text{s}^{-1}$  for *U. lactuca* (Israel et al., 1995). Gordillo et al. (2001) reported irradiance at 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$  as saturating for the net photosynthesis of *U. rigida*. In our experiments, light saturation ranged between 597.8 and 657.9  $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ , which is similar to that reported in the work of Gordillo et al. (2001) and higher than the stated values for *Ulva* sp. in the other studies. This may be due to the high irradiance at which they were maintained during the long term experiment, which was bearable due to a high nutrient supply. Figueroa et al. (2009) reported that nutrient-rich conditions, particularly nitrogen supply, can help *U. lactuca* withstand stressful conditions such as high irradiance and temperatures as it stimulates protection, through increased production of certain protective substances and accelerates the biochemical recovery of damaged structures (Peinado et al., 2004). It is worth mentioning that the highest photosynthesis rates were recorded at a light intensity higher than  $I_s$  (of 900  $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ ) with both nitrogen forms regardless of the concentration of nitrogen. Similarly, Henley (1993) reported that a considerable increase in photosynthesis occurs above  $I_s$ .

The significant high  $P_{max}$  at the nitrate concentration of 3.7 mM indicates that nitrogen was the limiting factor for photosynthesis at the temperature of 25°C and irradiance of 273  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . This implies that *Ulva* sp. can use nitrate at high concentrations, even higher than 3.7 mM, that is, 5 mM  $\text{NO}_3$  which is reported as the saturating concentration for *U. rigida* by Gordillo et al. (2001), for long periods (weeks) without any toxic effects being observed. Ammonium on the other hand was found to inhibit growth of *U. lactuca* at concentrations above 64.3  $\mu\text{M N/L}$  (Waite and Mitchell, 1972).

## Conclusion

A key finding of this study is that nitrate can be assimilated by *Ulva* sp. and yield similar or even higher growth rates than ammonium on the long term. Moreover, nitrate at a high concentration (3.7 mM) yielded a higher photosynthetic rate and improved photosynthetic efficiency as compared to the ammonium concentrations. This would have been reflected in a high RGR if reproduction had not been triggered.

Unlike ammonium, high concentrations of nitrate (up to 3.7 mM) are not toxic for *Ulva* sp. In fact, *Ulva* showed higher photosynthetic activity, which implies they may filter RAS effluent more efficiently than FTS effluent with long term exposure to high nutrient concentrations.

The  $P_{max}$  indicates that nitrate nitrogen was the limiting factor for photosynthesis at the concentration of 3.7 mM and at a temperature of 25°C and 273  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$

irradiance. This indicates that *Ulva* can filter nitrate even at higher concentrations than 3.7 mM, up to the saturation concentration.

Moving algae suddenly to higher nutrient concentrations triggers their intense reproduction faster than moving them to lower concentrations. Nitrate induced a fortnight pattern of reproduction, which is important for commercial-scale seaweed production.

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## Conflict of Interest

The authors declared there is no conflict of interest.

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