The effects of nitrate and phosphate on growth of algae, *Ulva rigida*

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Abstract

Nutrients especially nitrate and phosphate are the main limiting factors of algal growth in aquatic ecosystems. The present study was conducted to examine the effects of different concentrations of nitrate and phosphate simultaneously on growth of macro algae, *Ulva rigida*. For this purpose, algae were fed various concentrations of nitrate and phosphate during 28 days in 300 L tanks in the form of 4 experimental treatments with three replicates. The experimental treatments were: T_1: 10 mg L^{-1} NO_3+5 mg L^{-1} PO_4, T_2: 20 mg L^{-1} NO_3+10 mg L^{-1} Po_4PO_4, T_3: 30 mg L^{-1} NO_3+15 mg L^{-1} PO_4, T_4: 40 mg L^{-1} NO_3+20 mg L^{-1} PO_4 and one control treatment was considered. According to the results, all experimental treatments showed increased growth compared to the control treatment ($p<0.05$). There were no significant differences in growth of algae between T_3 and T_4 ($p>0.05$). The highest values of gain weight (88.02±0.74 g, nearly 17 times more than the initial weight) and daily growth percent (60.57±1.29 %) were observed in algae of T_3 and then T_4 (daily growth percent: 42.95±1.56 %). In conclusion, our results suggest that a concentration of 30 mg L^{-1} NO_3+15 mg L^{-1} PO_4 is more suitable for the growth of macro algae, *U. rigida*.

Keywords: Macro algae, *Ulva rigida*, Concentration, Nitrate, Phosphate

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Introduction

The green macro algae, *Ulva rigida,* belongs to the family Ulvaceae, a family with nearly 100 algae species (Shimada *et al*., 2003). The genus *Ulva* is found widely distributed throughout the costal habitats of the world including the coasts of Europe, North and South America, Africa, Asia and Australia. Also, this algae species is commonplace in the estuary regions of these areas (Sherrington, 2013). *Ulva* is known as the most important marine macro algae (Ale *et al*., 2011). In the coastal areas, some species of *Ulva* with fast growth rates are the cue of green tides (Buapet *et al*., 2008).

As aquaculture activities develop in coastal regions, the risk of disturbance to ecological sustainability, stress and diseases (such as white spot disease, a viral infection of penaeid shrimp) elevates due to the release of additional nutrients into aquatic ecosystems (reviewed by Costa-Pierce, 2010). Generally, algae have a high ability to absorb both toxic (such as unionized ammonia) and non-toxic nutrients and reduce the carbon dioxide and nutrient content (especially nitrate and phosphate) of surrounding waters and hence help in the stability of ecosystems (Ale *et al*., 2011). On the other hand, some macro algae such as *Ulva* have nutritional and pharmaceutical properties for human. *Ulva* efficiently consumes the polysaccharides that decreases the atherogenic index and finally total cholesterol in blood of human (Yasser and Samir, 2014). This macro algae has anti-cancer and anti-aging factors (Raja

et al., 2013) and also is used widely in food and cosmetic industries due to its high carbohydrate content. Also, *Ulva* is used as food for domestic animals including fishes in some modern aquaculture methods (Yasser and samir, 2014). In addition to these, *Ulva* is commonly used to improve the soil structure and quality of farmlands (Booth, 1969; Meland and Rebours, 2012). Urea had been used previously for the culture of macro algae, *Ulva fasciata* (Abkenar *et al*., 2004). Rouhi (2014) used *Ulva* to absorb and thus remove some nutrients including nitrate, phosphate and ammonium phosphate. The growth of *U. lactuca* was investigated in relation to various concentrations of NH_4Cl and $NaNO_3$ by Ale *et al*. (2011). Also, Kumari *et al*. (2013) examined the growth rate of *U. lactuca* in different concentrations of nitrate and phosphate. In a study by Buapet *et al*. (2008), the algal biomass and uptake of N and P by *U. reticulata* treated with added NO_3^-, NH_4^+, and HPO_4^{2-} were investigated in lab conditions. The growth and enrichment of *Chaetomorpha linum* treated with nitrogen and phosphorus based fertilizers were examined by Meńedez *et al*. (2002). Rabiei *et al*., (2014) suggested that *U. reticulata* can be used as a biological filter of released nitrogen compounds from shrimp farms due to its high ability in nutrient absorption. With regard to potentials of macro algae, the mass culture of them in ponds could be biologically and commercially useful. To this respect, the optimization of growth conditions of algae is an essential factor.

Therefore, in the present study, we examined the effects of the various concentrations of nitrate and phosphate on growth of *U. rigida* to find the optimum concentrations of these nutrients for growth.

Materials and methods

The *U. rigida* was collected from the intertidal zone of the Chabahar beach (latitude: 25°.17' N; longitude: 60°.37' E), Iran (Fig. 1). The selected healthy thalli were washed with seawater to remove epiphytic foreign matters following the removal of rhizoidal portions and then transported to the experiment place i.e. The Offshore Fisheries Research Center, Chabahar (OFRC) plastic tanks. In OFRC, the algae were held in a 300 l tank for 3 days (Kumari *et al.,* 2013).
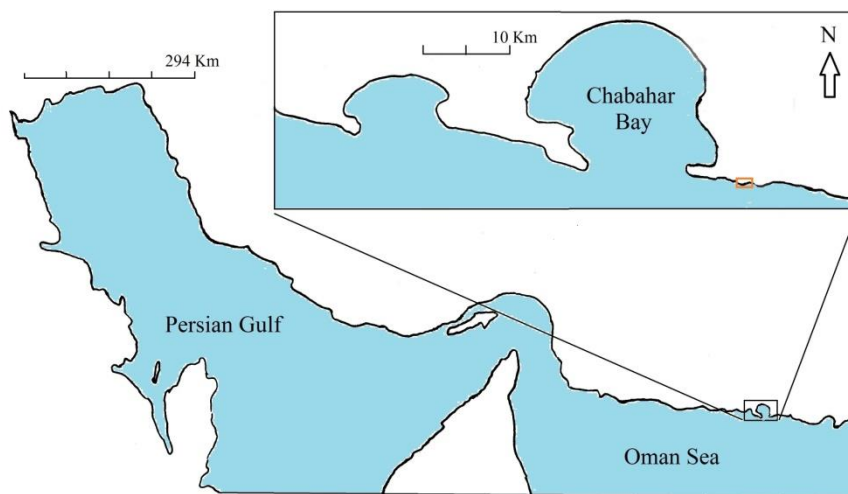

Figure 1: Map of the sample (*Ulva rigida*) collection.

In the present study, algae were fed various concentrations of nitrate and phosphate in 300 L tanks in the form of 4 experimental treatments with three replicates per treatment. The experimental treatments were: T_1: 10 mg L^{-1} NO_3+5 mg L^{-1} PO_4, T_2: 20 mg L^{-1} NO_3+10 mg L^{-1} PO_4, T_3: 30 mg L^{-1} NO_3+15 mg L^{-1} PO_4, T_4: 40 mg L^{-1} NO_3+20 mg L^{-1} PO_4 and a control was considered. The experiment was carried out for 28 days. 5 g of algae was weighed and placed in a basket into each of the experimental 300 L tanks (or each replicate). The tanks were held in natural sunlight (2500-3000 lux) and were provided gentle aeration using a central aerating pump. Also, to avoid much elevation of water temperature at noon, tanks were placed in a way that half of each tank's surface was covered by shade (Kumari *et al.,* 2013). The pH (8.48±0) and salinity (42.8±3.28 ppt) of water were monitored weekly and water temperature measured daily (27-32 °C) two times (morning (7:45:0) and afternoon (15:0:0)). The rearing medium was changed every other day to replenish the nutrient loss and nitrate and phosphate concentrations were regulated depending on kind of experimental treatment (Kumari *et al,.*

2013). For biometry of algae, the baskets containing algae were picked up and after shaking with hand (to remove additional water) placed on a clean piece of cloth for 15 min. then, the thalli of algae of each treatment were weighed using a digital balance (accuracy: 0.01 g). The weighing of algae was carried out on a weekly basis (Abkenar *et al*., 2004; Rabiei *et al*., 2014). Also, all the tanks were cleaned weekly after discharge of water.

The daily growth rate (%) of *U. rigida* was calculated according to following formula (Kumari *et al*., 2013):

$$DGR\% = [(W_t/W_0)^{1/t} - 1] \times 100$$

Where, W_t represents the fresh weight of algal thalli at time t and W_0 represents the initial fresh weight and t represents time in days (Kumari *et al*., 2013).

Statistical analysis
The SPSS software (version 16) was used for data analysis. The normality of data was investigated by Kolmogorov–Smirnov test. Because percentage data (% growth rate) did not have a normal distribution, proportional data were converted by angular transformation (arcsin $\sqrt{p}$). One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different (Meńedez *et al*., 2002; Buapet *et al*., 2008).

Results
According to ANOVA, algal growth showed significant differences between experimental groups ($p<0.05$). The algae of experimental treatments had significantly higher growth (g) compared to the control group over the course of the experiment (Figs. 2A, 2B, 2C, 2D; $p<0.05$). In this regard, the highest algal growth was observed in T_3 throughout the 28 days of the experiment (Figs. 2A, 2B, 2C, 2D; $p<0.05$). The biomass of algae until the end of the forth week increased, whereas the daily growth rate (%) decreased until the end of the third week and then increased significantly in the fourth week (except in T_1) (Fig. 3, Table 1; $p<0.05$). This increase was 15.71% for T_3.
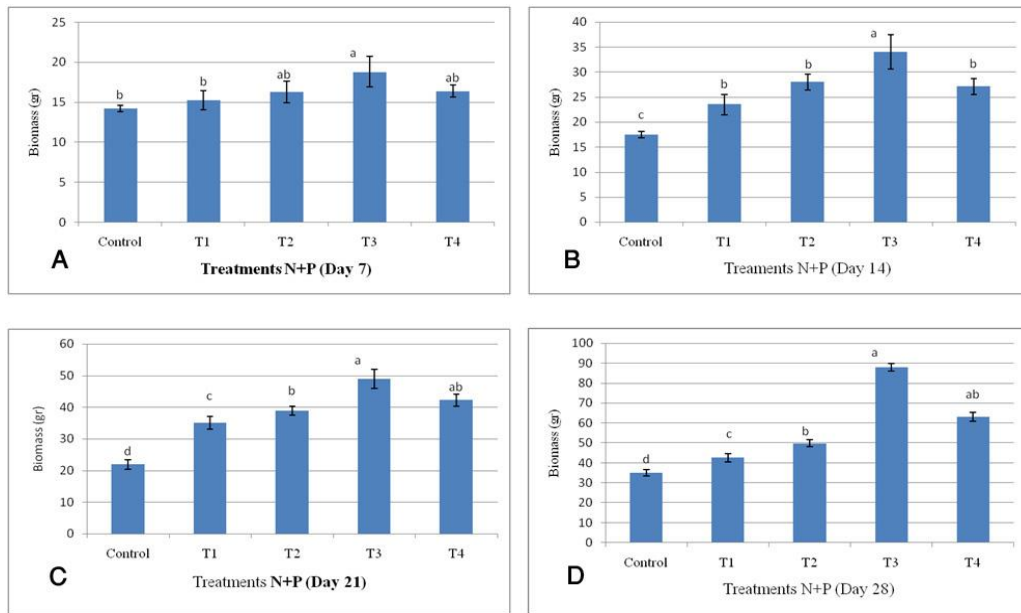
Figure 2: The growth (g) of *Ulva rigida* during four weeks culture in treatments of nitrate and phosphate (A: day 7; B: day 14; C: day 21; D: day 28). Bars are standard deviation and the significant differences were presented with different letters (*p*<0.05).
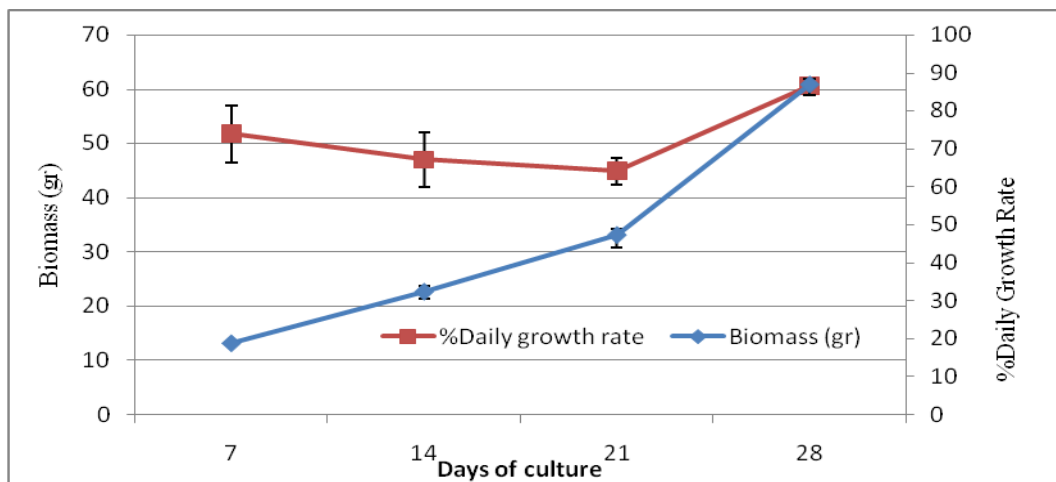
Figure 3: The biomass (g) and daily growth rate (%) of *Ulva rigida* over the 28 days treatment with nitrate and phosphate (30+15 mg L^{-1}). Bars are standard deviation.

Table 1: Daily growth rate (%) of *Ulva rigida* over the four weeks experiment.

Treatments / Weeks	Control	One	Two	Three	Four
First	39.79±0.65	41.57±3.10	44.31±3.67	51.76±5.22	44.74±3.21
Second	24.47±0.94	32.05±2.61	38.14±2.18	46.92±5.03	36.94±2.27
Third	20.41±0.95	31.87±1.66	35.30±1.30	44.86±2.44	38.39±1.76
fourth	24.44±0.63	29.0±1.37	33.85±1.20	60.57±1.29	42.95±1.56

Discussion

The appropriate concentration of nutrients especially nitrate and phosphate is essential to obtain optimum growth in algae species

(Harrison and Hurd, 2001). In the present study, our results demonstrated that nitrate and phosphate stimulate the growth of macroalgae, *U. rigida*. In this regard, a combination of 30 mg L^{-1}

NO_3+15 mg L^{-1} PO_4 had the most positive influence on growth. In this treatment, the mean weight of algae was 17 times more than the initial stocking weight. The *U. fasciata* cultured in a pond culture system (Bottome culture) did not show appropriate growth in spring while those cultured in the sea showed 5 times more growth compared to the initial stocking weight. However, the growth of *U. fasciata* decreased in autumn (Abkenar *et al.*, 2004). In a study on *U. rigida* and *Padina australi* (Rouhi, 2014), inspite of providing enough mineral fertilizers, less growth was obtained due to the use of old algae for the experiment. In that study, daily growth rate obtained was 7.87±2.34% on the 7th day. In our study, daily growth rate obtained was 44.31±3.67% in same period. Also the same researcher demonstrated that application the hen fertilizer in concentrations more than 20% decreses algal growth due to the increase in water turbidity and deposition of organic matter on algae. Usually the mineral absorbtion ability of younger algae is more than older ones (Harrison *et al.*, 1986). Meńedez *et al.* (2002) reported more biomass for N+P treatments of algae, *Chaetomorpha linum* compared to the control group. In our study, the daily growth rate (%) showed a declining trend till the third week of the experiment and then increased significantly (*p*<0.05). Similar results were found when the daily growth rate of *U. lactuca* increases with the increase in concentration of each nutrient up to 20 mg L^{-1} for nitrate and 10 mg L^{-1} for

phosphate, and thereafter it decreases gradually (Kumari *et al.*, 2013). Therefore, it seems that the growth of *U. rigida* in relation to nitrate and phosphate is dose-dependent since the growth of algae reduced when algae were fed nitrate and phosphate in concentrations less and more than 30 mg L^{-1} NO_3+15 mg L^{-1} PO_4. In another study the maximum growth rate (%77.38±5.70) was observed at the end of the third week when *U. rigida* was fed 20 mg L^{-1} NO_3 although this parameter decreased at higher concentrations (unpublished data). A maximum daily growth rate of 51.76±5.22 % was recorded for *U. rigida* in T_3 (for the first week) group of the present study that was three times more than the initial value. This growth was more than the results of Buapet *et al*. (2008) which may be due to the higher and apropriate concentration of nitrate in our study. In a study conducted by Buapet *et al*. (2008) a maximum daily growth rate of 15.1 % (compared to 51.76±5.22 in our study) was found for *U. reticulata* treated with 3.6 mg L^{-1} NO_3 for 7 days. Also, Rabiei *et al*. (2014) reported a daily growth rate of 1.6±0.1% for *U. reticulata* that was much less than our results in the present study. For *U. lactuca,* a daily growth rate of 17.43% and 4.26% was recorded in a recirculation system in summer and winter seasons respectively (Sherrington, 2013). In the present study, a daily growth rate of 60.57±1.29% obtained at the end of the fourth week of the experiment which was more than that of *U. lactuca* in the study of Sherrington, (2013). This result

seems to be due to the more appropriate concentrations of the nutrient in our study compared to the study of Sherrington (2013).

The differences in daily growth of algae, *U. lactuca* cultured in a medium containing ammonium chloride and sodium nitrate (50 μM N equal to 4.25 mg L^{-1}) was attributed to the nutrient concentration, age and size of the examined algae (Ale *et al.,* 2011). The above studies show that the growth rate of cultured macro algae is different depending on species of algae, age and size of algae, nutrient concentration, season and growth of non-target algae in the culture medium. Therefore, the optimization of growth condition for algae is species-specific. In our study, a daily growth rate of 60.57±1.29 % was found in T_3 group at the end of the experiment. Thus, a comparison with other studies shows that *U. rigida* is probably a fast-growing macro algae although this depends on experiment condition.

In our study, the daily growth rate (%) of *U. rigida* showed a declining trend till the third week of the experiment (due to increase temperature) and then increased significantly (except T_1). This may be due to the competition between algae for space, nutrient absorption, light and carbon dioxide resources (Harrison and Hurd, 2001). In conclusion, our results suggest that *U. rigida* is a fast growing macro algae and a concentration of 30 mg L^{-1} NO_3+15 mg L^{-1} PO_4 is more suitable for the mass-culture of this species.

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