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Time course nutrient uptake study of some intertidal rocky shore macroalgae and the limiting effect due to synergistic interaction

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The time course nutrient uptake study of some intertidal rocky shore macroalgae showed a marked variation in the uptake of nutrients. In the present study, *Caulerpa sertularioides, Padina tetrastomatica, Sargassum cinctum,* and*Gracilaria corticata* which are slow growingassimilated ammonium, whereas the fast growing *Chaetomorpha antennina, Ulva flexuosa,* and *Ulva lactuca* assimilated nitrate. Nitrate in the medium was assimilated quickly by these three species. It was below the detection level after five hours. The study showed that the characteristic of nutrient uptake varies between different macroalgae. The slow growing perennial macroalgae preferred ammonium and fast growing annuals preferred nitrate.The preference of different form of nitrogen is inferred to be mainly due to low energetic cost and opportunistic uptake. The combination of nutrient-N and phosphate in the medium showed a synergistic interactive effect in some of the algae wherein the uptake of nutrient was slowed comparatively. This synergistic interactive effect was significant ($P < 0.05$) and these were observed in *Caulerpa sertularioides*, *Chaetomorpha antennina*,and *Ulva lactuca*.

[Keywords: Ammonium; *Caulerpa sertularioides; Chaetomorpha antennina; Gracilaria corticata;* Nitrate; *Padina tetrastromatica;* Phosphate; *Sargasum cinctum; Ulva flexuosa; Ulva lactuca*]

Introduction

Macroalgae are the dominant producers in the intertidal rocky shore ecosystem, and it forms the basis of the food web[1-3,]. Macroalgae also play an important role in the socio-economic aspect of human welfare from food to medicine[3,4], and have a strong influence on the ecosystem function[5]. However, macroalgal community characteristics are influenced by a number of factors such as competition for space and light, protection from grazing, physical stress, growth, and reproduction[6,7].

Although the growth of macroalgae is affected by a range of factors namely temperature, light exposure, water velocity and day length[8,9]; availability of nutrient especially nitrogen significantly affects the macroalgal growth, abundance and community structure[10-12]. Nitrogen is the principal element that regulates algal metabolism. This often limits growth and increase in biomass as it is the key constituent of algal protein and enzymes[10,13-16]. Therefore, to obtain nutrients from the external environment either in high or low concentration is an important attribute in nutritional ecology[15].

Macroalgae have varied nutrient uptake characteristics, within and among species[17]. And the primary productivity of algae is significantly affected by the differential utilization of inorganic nitrogenous compounds[18]. Therefore, to understand the nutrient uptake characteristic between different macroalgae, a laboratory time course experiment was conducted to study the nutrient uptake in some intertidal rocky shore macroalgae. The subsequent results are discussed.

Materials and Methods

A group of macroalgae representing both annual (annual forms are normally fast growing species) and perennial form (slow growing) was selected for the present study. *Caulerpa sertularioides* (Gmelin) Howe (feathery), *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing (unbranched filaments), *Ulva flexuosa* Wulfen (Filamentous), *Ulva lactuca* L. (Leafy/thin sheet-like), *Padina tetrastomatica* Hauck (thick leathery), *Sargassum cinctum* C. Agardh (thick leathery), *Gracilaria corticata* (J. Agardh) J. Agardh (Coarsely branched) were used in the present study. Of these, *Chaetomorpha antennina*, *Ulva flexuosa*, *Ulva lactuca* are the annuals/seasonal algae. Algal samples were collected from Anjuna intertidal rocky shore - Goa, India (15°34’06” N, 73° 44’ 29’ E) during low tide. Macroalgal samples were then acclimatized in filtered aged seawater (low nutrient) overnight prior to the experiment.

A stock solution of 1 M each of Ammonium Chloride (NH_4Cl), Sodium Nitrate ($NaNO_3$), and Disodium Hydrogen Phosphate (Na_2HPO_4) (it will be referred as ammonium, nitrate, and phosphate hereafter) were prepared. The culture medium was prepared in filtered aged seawater (0.22 µm) by enriching with 306 µM (µM - micromole) of ammonium, 370 µM of nitrate and 103 µM phosphate. The high nutrient concentration used in the present study reflects the eutrophic condition observed during the seasonal nutrient surge. The nutrient concentration of filtered aged seawater was in the range of 0.43 – 3.36 µM. The final concentration was calibrated accordingly, and it was treated as the initial concentration.

A known weight of algae (1 g wet weight. l^{-1}) was put into a culture flask containing a fixed initial concentration of different nutrient treatment. Macroalgae were incubated in two different set of experiments. One set of experiment was ammonium, nitrate and phosphate alone, and in another set, ammonium and nitrate were combined with phosphate of the same volume to test the interactive effect. Control was maintained without algae. The culture was maintained under ca 55 µmoles.m^{-2}.sec^{-1} illumination at normal room temperature (24 ± 1) of 14/10 h light/dark cycle. The nutrient uptake was determined by the depletion of ammonium, nitrate, and phosphate in the medium. Samples of the media were collected at regular intervals [6, 12, 24, 48, 72 hours (h)]. However, nitrate uptake by *Chaetomorpha antennina*, *Ulva flexuosa*,and *Ulva lactuca* was very quick, and it was below the detectable level (BDL) after 6 h. So, the experiment was repeated with a reduced time (15, 30 min, 1, 2, 3, 4, 5 h) to measure the nitrate uptake. Accordingly, samples were collected to measure the depletion of nutrient/s due to assimilation and uptake. All tests were performed in triplicate. The nutrient concentration was analyzed using Autoanalyzer (Skalar, The Netherland).

The uptake rate was calculated from the relation: Uptake rate = $(N_1 – N_2)/(t_2 – t_1)$; where N_1 and N_2 are initial and final nutrient concentration, and t_2 and t_1 are the time interval. The significance of nutrient uptake was analyzed by repeated measure ANOVA, and the limiting effect between nitrogen and phosphate uptake by two-way ANOVA with the help of Statistica 8 software. The nutrient preference of the macroalgal species was inferred on the basis of the rate of disappearance from the medium.

Results

The nutrient depletion from the medium showed that the uptake of nutrient is species dependent (Fig. 1). Repeated measure ANOVA analysis showed that the uptake of nutrients by macroalgae was significantly affected by time (P<0.001). The depletion of nutrient from the medium showed that maximal uptake and assimilation of ammonium occurred mostly in *Caulerpa sertularioides, Padina tetrastomatica, Sargassum cinctum,* and *Gracilaria corticata.* Ammonium uptake by *Padina tetrastomatica* was highly significant (P < 0.001) (Fig. 1.2), and ~1.6 % of ammonium was detected in the medium after 6 h. Similarly, only 0.4 % of ammonium was recorded in the culture medium containing *Gracilaria corticata* after 12 h (Fig. 1.4).

Maximum nitrate uptake occurred in *Chaetomorpha antennina, Ulva flexuosa,* and *Ulva lactuca.* Nitrate in the medium was assimilated quickly by these species. Nitrate depleted in about five hours, subsequently, it was below the detectable level (BDL). However, ammonium and phosphate concentration was detected in the medium even after 72 h. *Ulva flexuosa* assimilated nitrate quickly from the medium, and it was below the detection level after 4 h (Fig. 1.6). Similarly, in the case of *Chaetomorpha antennina*and *Ulva lactuca* nitrate concentration in the medium was below the detection level after 5 h. In the absence of nitrate, the uptake of ammonium was recorded in these algae though the uptake time was over a period of 72 hours (Fig. 1; Table 1). In the entire experimental test, maximal phosphate uptake occurred in the initial stage (Fig. 1). The uptake of phosphate remained slow in the later part of the study.

There was a significant variation in the uptake rate of nitrate amongst different algal species. The uptake rate of nitrate was highest in three species (*Chaetomorpha antennina, Ulva fasciata,* and *Ulva flexuosa*) (Table 2). Highest was recorded in *Ulva flexuosa* (7.4 ± 1.3 µM. g^{-1} wet wt. h^{-1}) followed by *Ulva lactuca* (6.7± 1 µM. g^{-1} wet wt. h^{-1}). The lowest uptake rate was recorded in *Caulerpasertularioedes* and *Sargassum cinctum*(0.25 ± 0.04 µM. g^{-1} wet wt. h^{-1}). However, there was no significant variation in the uptake rate of ammonium and phosphate.

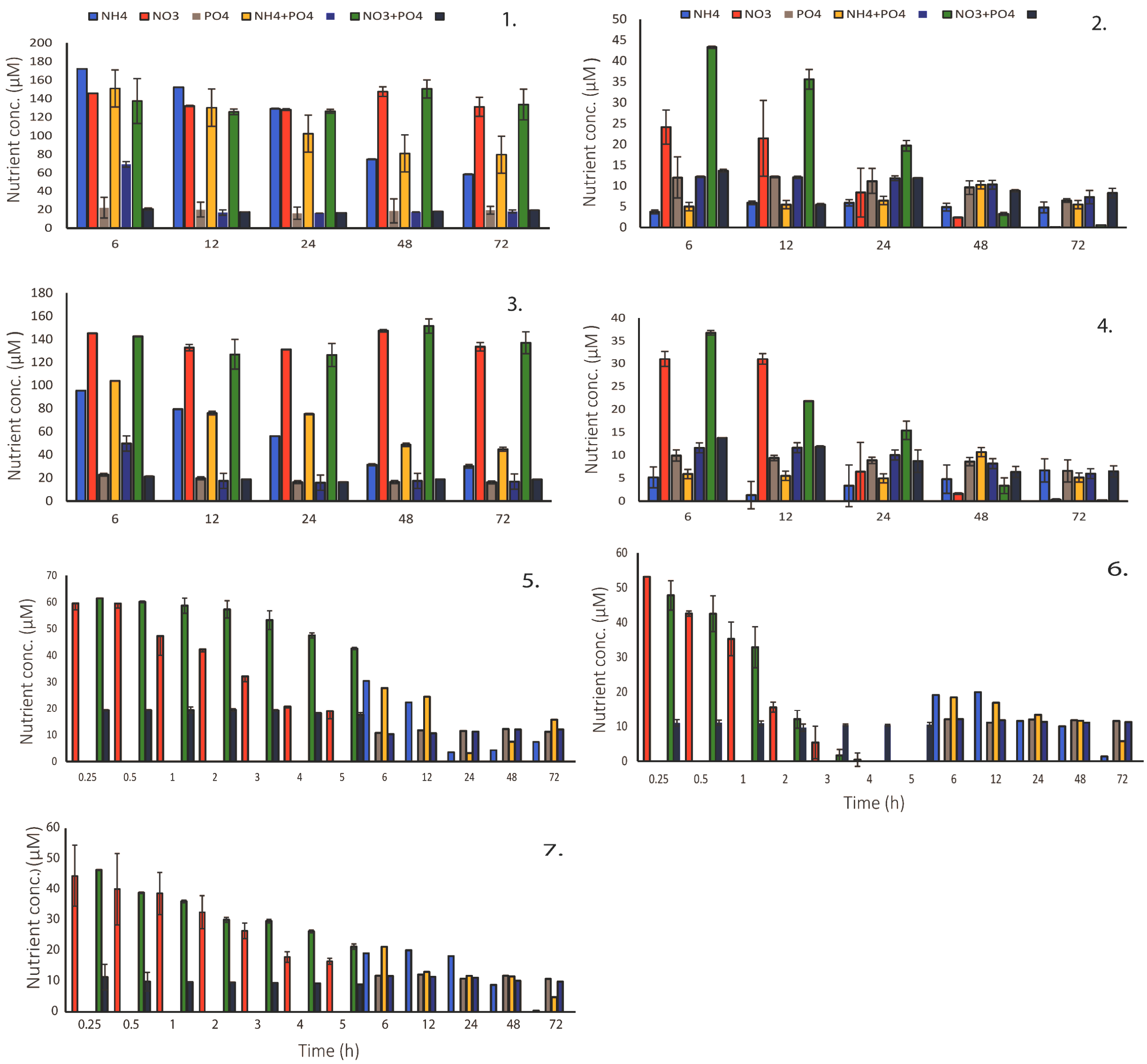

Fig. 1 — Nutrient concentration/s recorded after its depletion from the medium as a result of uptake and assimilation over a period of time course experiment by different macroalgae: 1. *Caulerpa sertularioides,* 2. *Padina tetrastromatica,* 3. *Sargassum cinctum,* 4. *Gracilaria corticata,* 5. *Chaetomorpha antennina,* 6. *Ulva flexuosa* and 7. *Ulva lactuca.* Nitrate-N uptake study duration was reduced to 5 hours for the species of *Chaetomorpha antennina, Ulva flexuosa* and*Ulva lactuca* due to fast uptake and assimilation. Error bar indicate the standard deviation. µM denotes micromoles.

Table 1 — Nutrient (ammonium, nitrate and phosphate) uptake rate of different macroalgae

	Uptake rate (µM. g^{-1} wet wt. h^{-1})		
Macroalgae	NH4	NO3	PO4
Caulerpa	0.2 ± 0.02	0.25 ± 0.04	0.09 ± 0.01
Chaetomorpha	0.31 ± 0.05	5.7 ± 0.8	0.1 ± 0.01
Ulva flexuosa	0.31 ± 0.05	7.4 ± 1.3	0.09 ± 0.01
Ulva lactuca	0.31 ± 0.05	6.7 ± 1	0.09 ± 0.
Padina	0.3 ± 0.06	0.4 ± 0.07	0.01 ± 0.01
Sargassum	0.24 ± 0.04	0.25 ± 0.04	0.09 ± 0.01
Gracilaria	0.33 ± 0.06	0.4 ± 0.07	0.1 ± 0.02

Comparison of uptake characteristic of different algae indicated a preference of nutrient-N. Species of *Caulerpa sertularioides*, *Padina tetrastomatica*, *Sargassum cinctum*, and *Gracilaria corticata* showed a preference for ammonium on the basis of nutrient depletion, whereas it was nitrate in the case of *Chaetomorpha antennina*, *Ulva flexuosa* and*Ulva lactuca.*

The study also showed the existence of a synergistic interactive effect on the nutrient uptake when nutrient-N (ammonium and nitrate) and phosphate were combined in the medium. This effect,

Table 2 — Nutrient concentration depleted during the time course (72 hours) uptake and assimilation by different algae. Combination of nutrient-N (ammonium and nitrate) and phosphate indicated limiting effect in the uptake of nutrient-N in many macroalgae.

	Nutrient concentration (μM)						
Macroalgae	NH4	NO3	PO4	NH4 + PO4		NO3 + PO4	
Caulerpa	245 ± 2	239 ± 1.4	84 ± 0.3	226 ± 1	85 ± 0.33	236 ± 1.5	84 ± 0.4
*Chaetomorpha**	298 ± 0.3	351 ± 2.6	92 ± 1	290 ± 2	91 ± 0.6	327 ± 1	85 ± 0.5
*Ulva flexuosa**	305 ± 2	370 ± 0	91 ± 0.5	300 ± 1	92 ± 0.4	370 ± 0	92 ± 1
*Ulva lactuca**	305 ± 0.5	353 ± 2	92 ± 0.3	301 ± 1	93 ± 1	349 ± 3	94 ± 0.6
Padina	298 ± 1	369 ± 0.8	97 ± 0.8	298 ± 2	94 ± 1	369 ± 1	95 ± 0.8
Sargasum	276 ± 1.5	236 ± 1.6	87 ± 0.5	261 ± 1.8	90 ± 0.3	233 ± 1	84 ± 0.3
Gracilaria	299 ± 1	369 ± 1	96 ± 1	302 ± 1	97 ± 1	369 ± 1	96 ± 0.8

*nitrate-N concentration was after 5 hours, while ammonium-N and phosphate concentration was after 72 hours. μM denotes micromoles.

although marginal, limited or slowed the uptake of nutrient-N. This limiting effect was detected in most of the macroalgae tested in varying degree (Table 1). However, a statistically significant (2-way ANOVA: $P< 0.05$) limiting effect on uptake due to the interaction of nitrate and phosphate was recorded only in *Caulerpa sertularioides*, *Chaetomorpha antennina*, and *Ulva lactuca*. Similarly, the limiting effect due to the interaction of ammonium and phosphate was significant (2 way ANOVA: $P> 0.05$) in *Caulerpa sertularioides*.

Discussion

The present study indicated that the nutrient uptake is mainly species dependent. There was a remarkable difference in the uptake characteristic amongst the macroalgae used in the study. It is apparent that the uptake of ammonium and nitrate is determined by the nutritional physiology of the macroalgae. Ammonium uptake was mostly observed in the slow growing perennial species of *Caulerpa sertularioides*, *Padina tetrastomatica*, *Sargassum cinctum*, and *Gracilaria corticata*. The ammonium uptake by *Padina tetrastomatic* and *Gracilaria corticata* was particularly fast compared to *Caulerpa sertularioides* and *Sargassumcinctum*. In addition, in all these species the uptake of ammonium was much faster than the nitrate uptake. This attribute was considered as the characteristic of nutrient-N preference. The preference of ammonium by algae was attributed to low energy cost in ammonium assimilation compared to nitrate assimilation[18]. In this manner, the algae don't have to expend energy on reducing the nitrate by direct utilization of ammonium. The rate of uptake of these macroalgae was also slow and it was in the range of 0.2 – 0.4 μM. g^{-1} wet wt. h^{-1}.Slow growing perennial macroalgae are also stated to develop a large nutrient storage pools which enable to effectively exploit the low nutrient condition in an environment[19]. Therefore, the low uptake rate complements the slow-growing nature of macroalgae.

Conversely, *Chaetomorpha antennina, Ulva flexuosa* and *Ulva lactuca* assimilated nitrate very quickly; similar nitrate depletion was also reported in *Ulva rigida*, and *U. intestinalis*[19,20]. The quick and high uptake capacity is inferred to make these macroalgae better adapted to deal with the nutrient surge. Quick uptake of nutrient by the species would be competitively superior. In agreement to this observation, the quick uptake and assimilation are stated to fulfil the nutrient demand that supports its fast-growing strategy[19]. These algae occur during monsoon, and during this period there is a significant increase (~46%) in nutrient concentration[21]. The high uptake capacity is believed to make these algae better adapted to deal with the seasonal nutrient surge and utilize the nutrient-N. This explains why these macroalgal species thrive in monsoon season.

The uptake rate was also highest in *Chaetomorpha antennina*, *Ulva flexuosa* and *Ulva lactuca*. The uptake rate of *Ulva flexuosa* was highest at 7.4 ± 1.3 μM. g^{-1} wet wt. h^{-1} followed by *Ulva lactuca* and *Chaetomorpha antennina*. This indicates that the annual species have developed a physiological strategy to deal with the seasonal nutrient surge and develop the biomass.The study also showed that ammonium can be utilized by these species in the absence of nitrate. However, the rate of uptake of ammonium was low and the uptake time was over a longer period.

The seasonal and opportunistic macroalgae have evolved to benefit from the seasonal nutrient pulses[22,23]. Reef et al.[11] also stated that high uptake of nitrogen takes place when nitrogen is limiting growth and its availability transient. However, it is stated that nutrient uptake being a plastic property, it becomes difficult to measure and compare among species because of intraspecific and experimental variation[15]. Some algae, also, do not show a saturation of nutrient uptake rate at a high environmental nutrient concentration[24].

Phosphate assimilated by the algae is mostly incorporated into phospholipids and polyphosphate molecules besides RNA[25]. It is also implicated in influencing the composition and algal succession in marine ecosystem[26]. In the present investigation, the maximal phosphate uptake occurred during the initial stage. The uptake of phosphate apparently reached a saturation point in the first six hours. No significant uptake occurred at a later stage, but a synergistic interactive effect on nutrient-N uptake due to the addition of phosphate was observed in some macroalgal species which resulted in limiting in the uptake of nutrient-N (ammonium and nitrate). The limiting effect due to this interaction was significant in *Caulerpa sertularioides*, *Chaetomorpha antennina*, and *Ulva lactuca*. The mechanism involved in the limiting effect due to synergistic interaction is not well understood, but the limiting effect occurred corresponding to uptake time. This effect slowed the uptake of nutrient-N and it is considered positive because this will allow the nutrient-N to remain available for longer period of time in the medium. Similar limiting effect was also reported in an earlier nutrient uptake studies[27,28] wherein nitrate uptake was limited or suppressed by ammonium. However, the knowledge of phosphate limiting and suppressing the uptake of nutrient-N is limited. We, therefore, suggested that some form of complexation must be taking place which result in the uptake limitation. On the other hand, the saturation of phosphate uptake also occurred in the early stage of incubation which might have a role in the limitation effect, because it is stated that the addition of phosphate increases the internal concentration of nitrogen[29]. Nevertheless, such limitation is considered as an important mechanism in mediating the effect of nutrient addition in the system[29-31].

This study showed that *Chaetomorpha antennina*, *Ulva flexuosa*, and *Ulva lactuca* have high uptake capacity of nitrate which can be potentially used as a phyco-remediation agent in cleaning up the nitrate enriched water bodies like aquaculture ponds. The high uptake capacity is believed to makes these algae better adapted to deal with the seasonal nutrient surge and utilize the nutrient-N. The present study further showed that the differential uptake behavior amongst the algae may be an attribute for better resource utilization which can eventually affect the macroalgal community structure.

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References

1 Graham, M.H., Effects of local deforestation on the diversity and structure of southern California giant kelp forest food webs. *Ecosys.*,7 (2004) 341-357.

2 Norderhaug, K., Christie, H., Fosså, J. & Fredriksen, S., Fish-macrofauna interactions in a kelp (*Laminaria hyperborea*) forest. *J. Mar. Biol. Assoc.* U.K. 85 (2005) 1279-1286.

3 Harley, C.D., Anderson, K.M., Demes, K.W., Jorve, J.P., Kordas, R.L., Coyle, T.A. & Graham, M.H., Effects of climate change on global seaweed communities. *J. Phycol.*48 (2012) 1064-1078.

4 Rönnbäck, P., Kautsky, N., Pihl, L., Troell, M., Söderqvist, T. & Wennhage, H., Ecosystem goods and services from Swedish coastal habitats: Identification, valuation, and implications of ecosystem shifts. *AMBIO: J. Human Environ.*,36 (2007) 534-544.

5 Ruiz, G.M., Fofonoff, P., Hines, A.H. & Grosholz, E.D., Non-indigenous species as stressors in estuarine and marine communities: Assessing invasion impacts and interactions. *Limnol. Oceanogr.*,44 (1999) 950-972.

6 Littler, M.M. & Littler, D.S., The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. *Amer. Natural.*,116 (1980) 25-44.

7 Littler, M.M., Taylor, P.R. & Littler, D.S., Algal resistance to herbivory on a Caribbean barrier reef. *Coral Reefs*,2 (1983) 111-118.

8 Peteiro, C. & Freire, Ó., Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of spain. *J.Appl.Phycol.*,25 (2013) 205-213.

9 Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C.M., Buck, B.H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S. & Karez, R., The genus *Laminaria* sensu lato: Recent insights and developments. *Eur. J. Phycol.*,43 (2008) 1-86.

10 Lapointe, B.E., Phosphorus-and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (rhodophyceae) in the Florida Keys: An experimental field study. *Mar. Biol.*, 93 (1987) 561-568.

11 Reef, R., Pandolfi, J.M. & Lovelock, C.E., The effect of nutrient enrichment on the growth, nucleic acid concentrations, and elemental stoichiometry of coral reef macroalgae. *Ecol. Evol.,* 2 (2012) 1985-1995.
12 Thacker, R., Ginsburg, D. & Paul, V., Effects of herbivore exclusion and nutrient enrichment on coral reef macroalgae and cyanobacteria. *Coral reefs,*19 (2001) 318-329.
13 Hecky, R. & Kilham, P., Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.,* 33 (1988) 796-822.
14 Fang, P., Zedler, J.B. & Donohoe, R.M., Nitrogen vs. Phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnol. Oceanogr.,* (1993) 906-923.
15 Hein, M., Pedersen, M.F. & Sand-Jensen, K., Size-dependent nitrogen uptake in micro-and macroalgae. *Mar. Ecol. Progr. Ser.,* 118 (1995) 247-253.
16 Wang, L. & Macko, S.A., Constrained preferences in nitrogen uptake across plant species and environments. *Plant, Cell Envir.,*34 (2011) 525-534.
17 Harrison, P.J. & Hurd, C.L., Nutrient physiology of seaweeds: Application of concepts to aquaculture. *Cah. Biol. Mar.,* 2001.
18 Dugdale, R.C., Wilkerson, F.P., Hogue, V.E. & Marchi, A., The role of ammonium and nitrate in spring bloom development in San Francisco bay. *Est. Coast. Shelf Sci.,* 73 (2007) 17-29.
19 Martinez, B., Pato L.S. & Rico, J.M., Nutrient uptake and growth responses of three macroalgae with perennial, opportunistic and summer-annual strategies. *Aqua. Bot.* 96 (2012) 14-22
20 Naldi, M. and Viaroli, P., Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po river delta, Italy). *J. Exp. Mar. Biol. Ecol.,*269 (2002) 65-83.
21 Anand, S.S., Sardessai, S., Muthukumar, C., Mangalaa, K., Sundar, D., Parab, S. & Kumar, M.D., Intra-and inter-seasonal variability of nutrients in a tropical monsoonal estuary (zuari, india). *Cont. Shelf Res.,*82 (2014) 9-30.
22 Chapman, A. & Craigie, J., Seasonal growth in *Laminaria longicruris*: Relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.,* 40 (1977) 197-205.
23 Raven, J.A. & Taylor, R., Macroalgal growth in nutrient-enriched estuaries: A biogeochemical and evolutionary perspective. *Water, Air and Soil Poll. Focus,*3 (2003) 7-26.
24 Harrison, P.J. & Hurd, C.L., Nutrient physiology of seaweeds: Application of concepts to aquaculture. *Cah. Biol. Mar.,* 2001.
25 Chopin, T., Lehmal, H. & Halcrow, K., Polyphosphates in the red macroalga *chondrus crispus* (rhodophyceae). *New phytol.,*135 (1997) 587-594.
26 Shang, S. & Hong, H., Study on phosphorus in Xiamen western sea. *Mar. Envir. Sci. Haiyang Huanjing Kexue Dalian,*15 (1996) 15-21.
27 Dortch, Q., The interaction between ammonium and nitrate uptake in phytoplankton. *Mar. Ecol. Prog. Ser.,* 61 (1990) 183-201.
28 Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M. & Kana, T.M., Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.,* 61 (2016) 165-197.
29 Bracken, M.E., Hillebrand, H., Borer, E.T., Seabloom, E.W., Cebrian, J., Cleland, E.E., Elser, J.J., Gruner, D.S., Harpole, W.S. & Ngai, J.T., Signatures of nutrient limitation and co-limitation: Responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos,*124 (2015) 113-121.
30 Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. & Smith, J.E., Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. lett.,*10 (2007) 1135-1142.
31 Harpole, W.S., Ngai, J.T., Cleland, E.E., Seabloom, E.W., Borer, E.T., Bracken, M.E., Elser, J.J., Gruner, D.S., Hillebrand, H. & Shurin, J.B., Nutrient co-limitation of primary producer communities. *Ecol. Lett.,*14 (2011) 852-862