Cryptogamie, Algol., 2005, 26 (4): 355-371

Seasonal variations in tissue nitrogen and phosphorus of eight macroalgae from a tropical hypersaline coastal environment

Sergio O. LOURENÇO[a], Elisabete BARBARINO[a,b], Andyara NASCIMENTO[a,c] & Rodolfo PARANHOS[c]*

[a] *Departamento de Biologia Marinha, Universidade Federal Fluminense, Caixa Postal 100644, CEP 24001-970, Niterói, RJ, Brazil*

[b] *Programa de Pós-Graduação em Biotecnologia Vegetal, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-900, Rio de Janeiro, RJ, Brazil*

[c] *Instituto de Biologia, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-900, Rio de Janeiro, RJ, Brazil*

(Received 25 January 2002, accepted 13 December 2004)

Abstract – Araruama Lagoon is a coastal hypersaline environment in Southeastern Brazil with a tropical climate. The Lagoon is connected to the sea by a single narrow channel, near which fluctuations in salinity occur over a wide range. Total tissue nitrogen and phosphorus of *Acanthophora spicifera*, *Chaetomorpha crassa*, *Derbesia vaucheriaeformis*, *Gracilaria cervicornis*, *Gracilariopsis tenuifrons*, *Hypnea valentiae*, *Rhizoclonium africanum*, and *Ulva lactuca* from the transient environment between the Lagoon and the sea were analysed during two seasonal cycles, from autumn 1997 to autumn 1999. Almost all species showed great variations in the N, P and N:P values over the period. Similar patterns of seasonal fluctuations of tissue N and P during the two years of sampling were found, suggesting the occurrence of common cyclic annual variations. Mean percentages of tissue nitrogen (calculated from data of all species combined) attained maximum values in autumn and minimum in spring, varying from 3.25% to 4.80% of the dry weight (d.w.). Peaks of tissue phosphorus did not coincide with N peaks; maximum mean values of tissue P were obtained in summer (*ca* 0.40% of d.w.) and minimum values in spring (*ca* 0.24% of d.w.). During autumn, winter and spring mean atomic N:P ratios were around 30:1, while the summer values were significantly lower (19.2:1 and 23.9:1 in 1998 and 1999, respectively). The analysis of dissolved nutrients showed low concentrations of inorganic phosphorus (except in summer), agreeing with the predominance of the relatively high N:P ratios measured in the tissues. The shallow waters of the sampling site show characteristics of an oligotrophic to mesotrophic environment, and despite ongoing eutrophication levels of dissolved phosphorus seem to control increments in macroalgal biomass.

Dissolved nutrients / hypersaline coastal lagoon / macroalgae / tissue nitrogen / tissue N:P ratio / tissue phosphorus

Résumé – **Variations saisonnières de l'azote et du phosphore des tissus de huit macroalgues provenant d'un milieu côtier tropical hypersalin**. Le Lagon de Araruama est un milieu hypersalin sous l'influence d'un climat tropical humide, au sudest du Brésil. Ce lagon est relié à la mer par un chenal étroit, où la salinité est très variable. L'azote et le phosphore

* Correspondence and reprints: solourenco@yahoo.com
Communicating editor: Olivier De Clerck

totaux du tissu (N_{tissu} et P_{tissu}) des algues *Acanthophora spicifera*, *Chaetomorpha crassa*, *Derbesia vaucheriaeformis*, *Gracilaria cervicornis*, *Gracilariopsis tenuifrons*, *Hypnea valentiae*, *Rhizoclonium africanum*, et *Ulva lactuca*, provenant de l'interface lagon-mer, ont été analysés pendant deux cycles saisonniers, de l'automne 1997 à l'automne 1999. Presque toutes les espèces présentent de grandes variations en N, P et du rapport N:P pendant cette période. Des variations saisonnières similaires des N et P tissulaires ont été observées durant la même période d'échantillonage, ce qui suggère un cycle annuel de variations commun. Le pourcentage moyen de l'azote tissulaire (calculé à partir de l'ensemble les données) a atteint des valeurs maximales en automne et minimales au printemps, de 3,5 % à 4,80 % du poids sec (p.s.). Les pics de phosphore tissulaire ne correspondent à ceux de l'azote. Les valeurs maximales de phosphore tissulaire ont été obtenues en été (0,40 % p.s.) et les valeurs minimales au printemps (0,24 % p.s.). Pendant l'automne, l'hiver et le printemps, la moyenne du rapport atomique N:P s'établit autour de 30:1, alors que les valeurs de l'été sont significativement moindres (19,2:1 et 23,9:1 en 1998 et 1999 respectivement). L'analyse des nutriments dissous a montré des concentrations très basses du $P_{inorg.}$ (sauf en été), en accord avec la prédominance des rapports N:P relativement élevés mesurés dans les tissus. Les zones peu profondes des sites d'échantillonage présentent des caractéristiques d'un environnement oligotrophe à mésotrophe, et en dépit d'une eutrophisation en cours, le niveau du phosphore dissous semble contrôler l'augmentation de la biomasse des macroalgues.

Nutriments dissous / lagon hypersalin côtière / macroalgues / azote du tissu / rapport N:P de tissu / phosphore de tissu

INTRODUCTION

Growth of macrophytes and phytoplankton in tropical coastal waters is generally limited by nutrient availability (Lapointe & Duke, 1984). Urbanisation of coastal areas has greatly increased the inputs of nitrogen and phosphorus into many aquatic systems, with resultant impacts at the population and ecosystem level (Valiela *et al.*, 1990). Increased abundance of nuisance macroalgae is among the direct consequences of nutrient loading. The macroalgae that proliferate under these circumstances may become important primary producers in coastal embayments or may outcompete preferred species (Rivers & Peckol, 1995). Opportunistic algae are capable of uptake, assimilation, and storage of large amounts of nitrogen in areas of high N loading, resulting in low water-column concentrations of nutrients (Peckol *et al.*, 1994), mainly in shallow environments.

Monitoring of water-column nutrient concentrations is the traditional method used to indicate nutrient enrichment, and effects on the primary producers are inferred. However, monitoring the concentration of total N and P in the tissue of macroalgae may be a more useful indicator of enrichment or eutrophication potential (Fong *et al*, 1994). In particular, tissue N of the algae is a better indicator of N supply than any other estimate of growth or biomass accumulation (Fong *et al.*, 1998). Macroalgae respond to nutrient enrichment by taking up nutrients, growing, and storing "excess" nutrients for future growth (Hanisak, 1979; Fujita, 1985; Björnsäter & Wheeler, 1990). Thus, total nutrient concentration in the algal tissue integrates the nutrient regime over some period of time (Wheeler & Björnsäter, 1992). Results of both modelling and experiments suggest that foliose green macroalgae may be especially useful indicators for assessing nutrient enrichment as they are opportunists with very fast nutrient uptake and growth rates, as well as a large internal storage capacity for nutrients (Duke *et al.*, 1989).

Monitoring of tissue nutrients to detect enrichment could occur at less frequent intervals than monitoring of the water-column nutrients, and allows a more accurate evaluation of the nutrient status of the macroalgae. A sequential enrichment/starvation study demonstrated that, following enrichment, internal stores of nutrients within the tissue of *Enteromorpha* spp. were not depleted for up to 10 days (Fujita, 1985). A simulation model of *Enteromorpha* spp. from lagoons in southern California suggests that the time lag between nitrogen uptake and growth may be as long as 20 days (Fong *et al.*, 1994).

Studies on tissue N and P content of macroalgae have been carried out predominantly in temperate (Wheeler & Björnsäter, 1992; Peckol *et al.*, 1994), warm temperate (Fong *et al.*, 1993) and subtropical coastal environments (Hanisak, 1993). Those studies have shown wide fluctuations in the tissue content of N and P, which could be related to changes in climate and nutrient availability during the year. In contrast, there are few reports on tissue N and P of algae from tropical environments (Schaffelke, 1999; Fong *et al.*, 2001) and virtually no information on macroalgae from hypersaline coastal lagoons.

The Araruama Lagoon, Brazil (22.7°-23.0°S, 42.0°-42.4°W) lies parallel to the coast of Rio de Janeiro State (Fig. 1) and is connected to the sea by a single narrow channel, which dramatically dampens the water exchange. There is a negligible river runoff relative to the large volume, making the lagoon one of the largest permanently hypersaline lagoons of the world (Kjerfve *et al.*, 1996).

The macroalgal flora of Araruama Lagoon is composed of 98 species, and the majority occur in areas strongly influenced by the sea. In the inner areas of the Lagoon, species tend to attain lower and extremely variable biomass (Reis & Yoneshigue-Valentin, 1996). Local macroalgae have to respond to extreme environmental conditions resulting from the hypersalinity, high temperatures and reduced availability of hard substrata. These characteristics make the Araruama Lagoon a unique system, deserving specific studies to clarify its dynamics. Considering the local climatic stability, we hypothesised that the seaweeds of Araruama Lagoon would show no significant variations in their contents of tissue N and P throughout the year. The local flora would show a different pattern compared to seaweeds of temperate environments, which typically show seasonal variations in their tissue components.

In this study we report the seasonal variations of nitrogen, phosphorus and N:P atomic ratio of the most abundant macroalgal species of the Araruama Lagoon. We establish relationships among the trends observed in algal N and P contents and the concentrations of dissolved nutrients in the system, variations in salinity, and physical characteristics of the sampling site.

MATERIALS AND METHODS

Study site

Araruama Lagoon is the largest hypersaline coastal lagoon of Brazil, measuring 210 km^2 (in addition to 65 km^2 of adjacent salt-producing ponds), a mean depth of 3.0 m (maximum depth of 17 m), and a water volume of 0.618 km^3 (Fig. 1). As a result of a semiarid climate and a negative water balance (evaporation: precipitation balance = 1.3:1), Araruama Lagoon is one of the largest permanently hypersaline coastal lagoons in the world, with a mean salinity of 52 psu (Kjerfve *et al.*, 1996). The lagoon is connected to the ocean via a single 14-km long

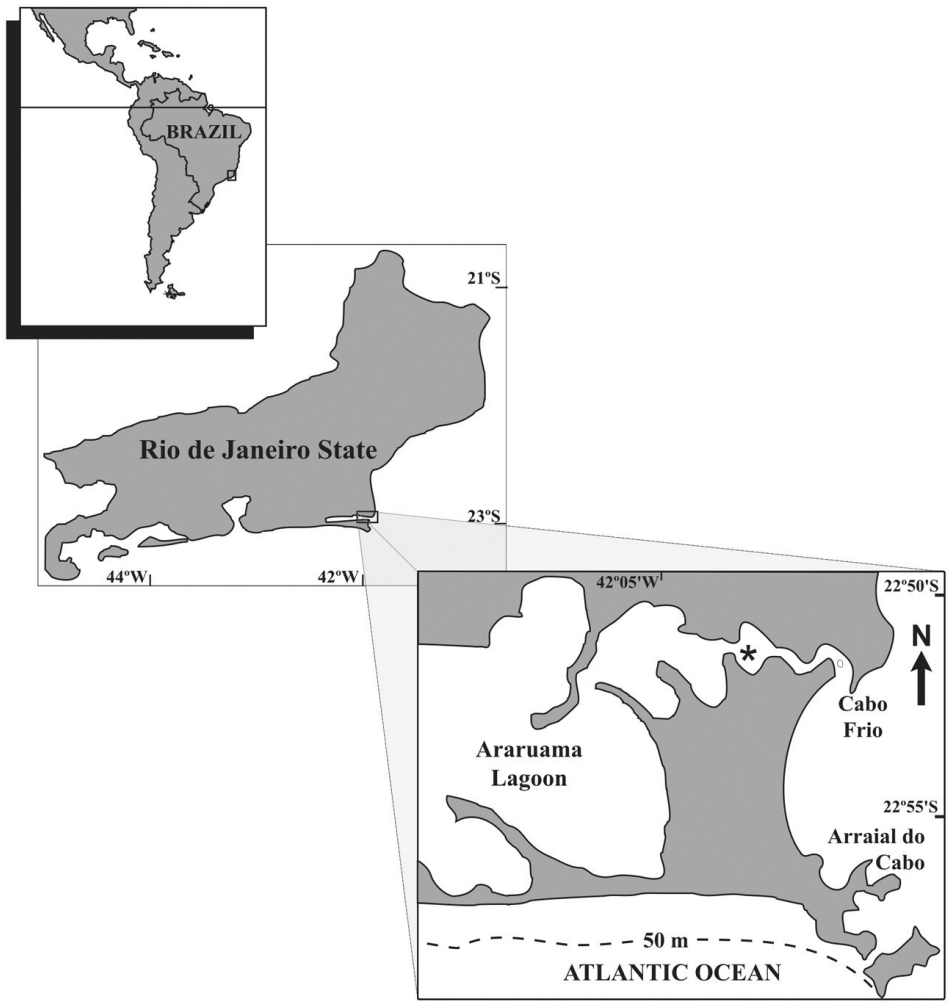

Fig. 1. Sampling site in the study area. * = Sampling site.

channel, "canal de Itajuru", which crosses a town, Cabo Frio. The current human population in the lagoon drainage basin is 200,000 and varies seasonally and during weekends, since the lagoon region is a major recreation area. In summer, the region receives more than one million tourists. Virtually all wastewater of the region drains to the lagoon without treatment (Kjerfve *et al.*, 1996). The sandy sediments between 0 and 3 m water depths are rich in carbonates and relatively poor in organic carbon. Abundant deposits of 2-4 cm-shells in length of the bivalve *Anomalocardia brasiliensis* are found in the surface sediments (Baeta Neves, 1983). The shells serve as a favourable substrate for biofilms and patchily distributed microphytobenthos populations with a mat thickness of 1 to 3 mm (Knoppers *et al.*, 1996). The microphytobenthos consists mainly of cyanobacteria, and represents the dominant autotrophs of the Araruama Lagoon (Baeta Neves, 1983).

Algae studied

In this study eight macroalgal species were analysed. The identification of the macroalgae was carried out following the checklist of Wynne (1998).

Green algae: *Derbesia vaucheriaeformis* (Harv.) J. Agardh (Order Bryopsidales), *Chaetomorpha crassa* (C. Agardh) Kütz., *Rhizoclonium africanum* Kütz. (Order Cladophorales), and *Ulva lactuca* L. (Order Ulvales).

Red algae: *Acanthophora spicifera* (Vahl) Børgesen (Order Ceramiales), *Gracilaria cervicornis* (Turner) J. Agardh, *Gracilariopsis tenuifrons* (C.J. Bird & E.C. Oliveira) Fredericq & Hommers. (Order Gracilariales), and *Hypnea valentiae* (Turner) Mont. (Order Gigartinales).

Sampling

Sampling began in June 1997 and continued through June 1999 (austral autumn). The samples were collected seasonally for a total of 9 events, and each event occurred in the last 3 weeks of each season. The sampling site was located near the Itajuru Channel (Fig. 1), where both the number of species and macroalgal biomass are maximum in the Lagoon (Reis & Yoneshigue-Valentin, 1996). That part of the Lagoon is subject to more oceanic influence, which includes small tidal variations and lower salinity. The sampling site corresponds to a transitional environment between a hypersaline lagoon and the sea, but salinity is predominantly higher than 40 psu. Except for *U. lactuca*, which often floats, the other species are found either attached to the few small rocks of the sampling site or partially buried in the sediment (water column between 0.4 to 0.8 m).

Whole thalli of adult plants were collected in the early morning and washed in the field with the water of the Lagoon, in order to remove epiphytes, sediment and detritus. At least 10 whole plants of each species were collected at each site, independent of the size of individual plants. All species were typically found at the same specific points in the site throughout the study (e.g. *D. vaucheriaeformis* was sampled always at the same rocks near the Itajuru Channel; *Chaetomorpha crassa* was found always partially buried in the sediment close to a small artificial creek located in the south area of the sampling site). The plants were packed in plastic bags and kept on ice until return to the laboratory (less than one hour). In the laboratory, samples were gently brushed under running seawater, rinsed with distilled water, and dried at 60°C for at least three days and until constant weight. The dried material was ground into a powder and kept in desiccators containing silica-gel at room temperature until the chemical analysis. At the time of each collection of macroalgae, four 250 ml-water samples (n = 4) for dissolved nutrient analysis were taken from 15 - 20 cm below the water surface, as well as measurements of local temperature at the same depth. The samples of water were filtered (0.45 μm pore) and kept at –20°C for spectrophotometric determinations of ammonia, nitrate, and nitrite (Parsons *et al.*, 1984), phosphate and urea (Grasshoff *et al.*, 1983).

Tissue analysis

Total N and P were determined in algal tissue after peroxymonosulfuric acid digestion, using a Hach digestor (Digesdhal®, Hach Co.) (Hach *et al.*, 1987). This was probably the first time that this method was used with macroalgal samples. Samples containing 50 to 200 mg (dry matter) were digested with 4 ml concentrated sulphuric acid (Merck Co.) at 440°C, for 3 minutes (or until visible destruction of the material). After this, 17 ml of 30% hydrogen peroxide

(Merck Co.) were added to the sample at a flux of 3.0 ml per minute, using a capillary funnel. Samples were boiled for one minute more after the complete addition of hydrogen peroxide, in order to evaporate the residual hydrogen peroxide. In these digestion conditions all nitrogen present in a sample is converted to ammonia, and phosphorus is converted to phosphate. Samples were cooled at room temperature and diluted with ultra-pure water (Milli-Q® water) to 100 ml. For nitrogen analysis, 2.5 ml of the diluted samples were collected and diluted to 12.5 ml and reacted with polyvinil alcohol® (2 drops), mineral stabilizer® (2 drops), and Nessler reagent® (0.5 ml) (Hach Co.). For phosphorus analysis, 1.0 ml of the diluted samples was collected and diluted to 25 ml with ultra-pure water and reacted with PhosVer phosphate reagent® (Hach Co.). The use of digestion and analysis of nitrogen after Hach *et al.* (1987) yields the same results for a large set of samples (pure proteins, amino acid standards, manure, vegetables, meat, liquid samples, etc.) compared to the traditional Kjeldahl method (Watkins *et al.*, 1987). The use of the Hach procedures has the advantage of allowing the use of the same digested sample for other analysis, such as total phosphorus. After digestion the sample is stable for N analysis for 28 days and for 24 hours for phosphorus analysis, if kept at 2-8°C.

For each species in each sampling period at least four independent (from different plants) measurements of tissue N and P were performed, but in some cases five or six analyses were done ($4 \leq n \leq 6$).

Statistical analysis

The results for each species separately and for total measurements for all species combined were analysed by single-factor analysis of variance (ANOVA) with significance level $\alpha = 0.05$ (Zar, 1996) followed, where applicable, with a Tukey's multiple comparison test. Time was the only factor considered in ANOVA.

RESULTS

Mean salinity ranged from 44.0 to 51.5 (Tab. 1). No clear relationship between salinity and seasons was found.

Among the dissolved nutrients analysed, nitrite showed the lowest concentrations, with an overall mean value of 0.36 ± 0.17 µM. Dissolved urea showed minor variations in the measurements throughout the two years of sampling, with a mean value of 0.57 ± 0.17 µM. During the first year of sampling (autumn 1997 – summer 1997/98) nitrate concentrations showed minor fluctuations, varying around 1.5 µM; however, in the second year of sampling, nitrate levels attained the maximum (winter and spring 1998, around 3.1 µM) and the minimum (autumn 1998 and summer 1999, around 0.60 µM) values measured during the study. Ammonia and phosphate showed similar trends of variation, with stability in the concentrations from autumn to spring, and peaks in summer. Total dissolved nitrogen reached higher values in summer. The N:P ratio of the dissolved components in water showed high values in spring due to low concentrations of phosphate, and lower values in summer due to high concentrations of ammonia (Tab. 1).

Nitrogen and phosphorus content and N:P ratio varied greatly over time in the algal tissues. Algae tended to attain maximal percentage of tissue nitrogen in autumn, and minimal values in spring. This general description is exemplified by *U. lactuca*, *G. tenuifrons* and *H. valentiae* (Fig. 2A), visually the three most

Table 1. Measurements of salinity and dissolved nutrients at the sampling site in Araruama Lagoon. Data represent the mean ± standard deviation (n = 4) and are expressed as μM, except for salinity (psu) and N:P ratio (no units).

Parameter	*Autumn 1997*	*Winter 1997*	*Spring 1997*	*Summer 1998/1999*	*Autumn 1998*	*Winter 1998*	*Spring 1998*	*Summer 1998/1999*	*Autumn 1999*
Salinity	47.5 ± 0.7	46.3 ± 0.3	44.0 ± 0.41	44.9 ± 0.8	50.5 ± 0.6	44.8 ± 0.6	51.5 ± 0.4	45.5 ± 0.7	46.3 ± 0.5
$N - NO_3^-$ (nitrate)	1.6 ± 0.22	1.58 ± 0.24	1.4 ± 0.21	1.3 ± 0.06	0.58 ± 0.11	3.1 ± 0.16	3.1 ± 1.0	0.61 ± 0.18	2.4 ± 0.65
$N - NO_2^-$ (nitrite)	0.67 ± 0.15	0.30 ± 0.00	0.25 ± 0.06	0.23 ± 0.06	0.30 ± 0.05	0.21 ± 0.05	0.21 ± 0.04	0.50 ± 0.09	0.54 ± 0.13
$N - NH_3$ (ammonia)	4.1 ± 0.40	3.1 ± 0.95	3.5 ± 1.0	8.0 ± 1.9	3.8 ± 0.72	3.4 ± 0.85	2.3 ± 0.59	8.9 ± 0.60	4.1 ± 0.56
$N - CO(H_2N)_2$ (urea)	0.41 ± 0.14	0.48 ± 0.05	0.46 ± 0.10	0.89 ± 0.23	0.79 ± 0.24	0.55 ± 0.15	0.59 ± 0.14	0.38 ± 0.07	0.53 ± 0.08
$P - PO_4^{3-}$ (phosphate)	0.61 ± 0.02	0.58 ± 0.14	0.21 ± 0.09	0.72 ± 0.22	0.53 ± 0.08	0.68 ± 0.22	0.18 ± 0.10	2.9 ± 0.65	0.91 ± 0.27
Total dissolved N	5.2 ± 0.39	3.8 ± 0.96	5.6 ± 1.1	10.4 ± 2.4	5.2 ± 1.0	7.3 ± 1.1	6.2 ± 1.6	10.4 ± 0.33	7.6 ± 1.6
Dissolved N:P ratio	16.5 ± 0.84	6.5 ± 1.1	26.9 ± 2.3	14.5 ± 2.56	9.8 ± 1.3	10.7 ± 0.90	34.4 ± 2.1	3.7 ± 1.0	8.3 ± 1.9

abundant species during the sampling events. *A. spicifera* showed a high degree of variation in N content, from 3.0 ± 0.2% in spring 1998 to 6.7 ± 0.3% in winter 1997, the highest tissue N concentration measured in this study (Tab. 2). On the other hand, *D. vaucheriaeformis* had consistently high concentrations of tissue N, with and small variations ranging between 4.6 and 6.5% of d.w.

The tissue N content of all species were significantly higher in autumn (ANOVA, $F_{8,278} = 7.26, p < 0.001$) (Fig. 2B); mean values of tissue N were similar during the other seasons (ANOVA, $F_{5,188} = 1.58, p = 0.17$), except in spring (lower mean values). The comparison of Figs 2A and 2B makes clear that variations in tissue N of the three dominant species are extremely similar to general trends reported for mean values of all macroalgae at the sampling site, based on the mean values for tissue in the eight species studied. It may indicate a strong influence of these three species on the general trends observed.

Tissue P showed a greater variation among the species than the values of tissue N, ranging from 0.12 ± 0.01% (*U. lactuca*, spring 1998) to 0.56 ± 0.04% (*H. valentiae*, autumn 1998).

Overall trends of P concentrations in tissues were different to those observed for tissue N. For almost all species, high values of tissue P were measured in summer and autumn and lower values in winter and spring (ANOVA, $F_{8,256} = 19.5, p < 0.001$) (Fig. 3A and 3B, Tab. 2). From January (summer) to June (end of autumn) the algae seemed to accumulate high concentration of P. Peak values of tissue P occurred in summer 1998 (March), which values were statistically higher than all other mean values obtained in autumn and summer 1999 (ANOVA, $F_{4,137} = 6.90, p < 0.001$). During the following winter and spring algae showed lower and less variable values of tissue P (ANOVA, $F_{3,119} = 0.40, p = 0.75$) (Fig. 3B).

R. africanum showed the highest N:P ratio (45.1 ± 3.1, autumn 1999), while *U. lactuca* showed the lowest (13.9 ± 1.52, summer 1998) among all species (Tab. 2). *G. tenuifrons* and *U. lactuca* tended to show similar values of N:P ratio over the study (Fig. 4A). Mean values for all macroalgae in each sampling were almost constant (around 30:1) from autumn to spring (ANOVA, $F_{6,203} = 0.58$, $p = 0.75$). In summer, significantly lower mean N:P ratios were recorded (19.2:1 and 23.9:1 in 1998 and 1999, respectively) (ANOVA, $F_{8,251} = 7.77, p < 0.001$) (Fig. 4b).

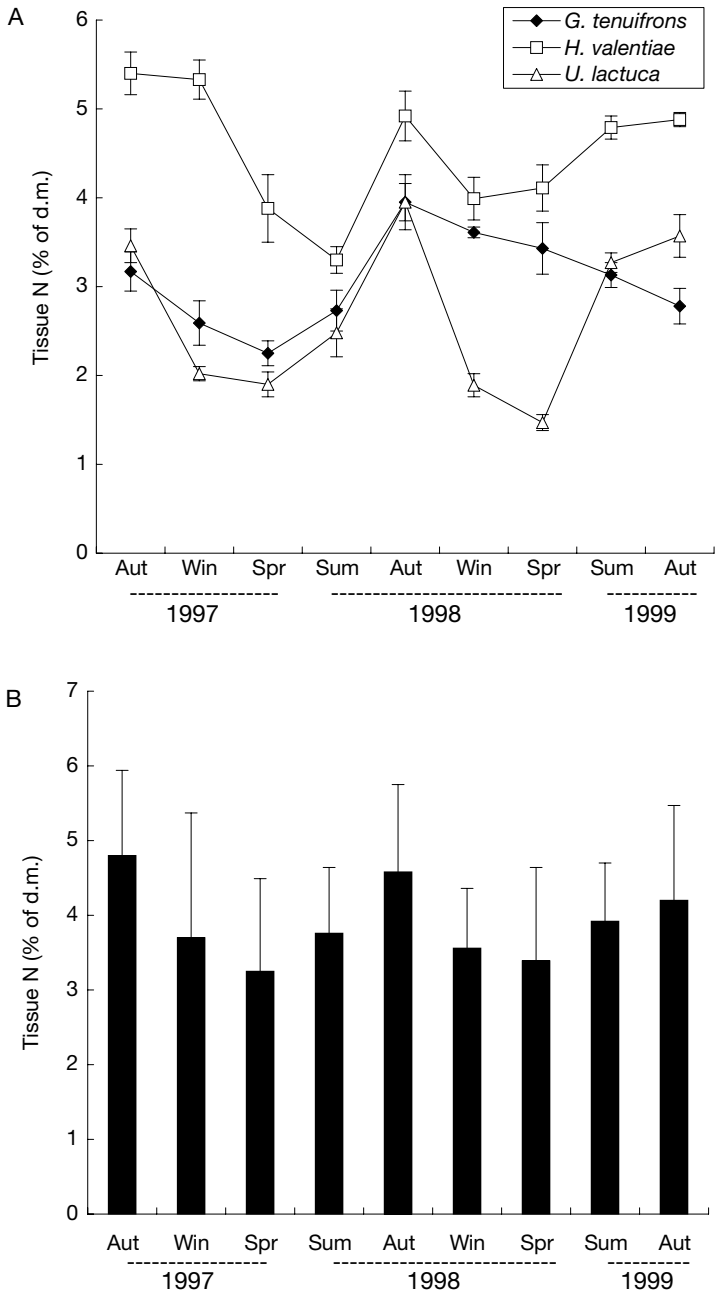

Fig. 2. Seasonal fluctuations in the content of nitrogen of *Gracilariopsis tenuifrons*, *Hypnea valentiae*, and *Ulva lactuca* (A), and mean values of N in the tissues of all macroalgae (B) collected in nine seasonal sampling in the field. Data are expressed as percentage of the dry weight (d.w.). In (A) each point represents the mean of four to six replicates ± standard deviation ($4 \leq n \leq 6$). In (B) each bar represents the mean of 32 to 44 measurements ± standard deviation ($32 \leq n \leq 44$).

Table 2. Maximum and minimum values of tissue N, P and N:P atomic ratio of eight species of macroalgae from Araruama Lagoon, in nine seasonal sampling. Tissue N and P are expressed as percentage of the dry weight, except for N:P atomic ratio (no units). Data represent the mean of four determinations ± standard deviation (n = 4).

	Tissue N		*Tissue P*		*Tissue N:P*	
	Maximum	*Minimum*	*Maximum*	*Minimum*	*Maximum*	*Minimum*
A. spicifera	6.7 ± 0.3 Winter 1997	3.0 ± 0.2 Spring 1998	0.44 ± 0.04 Summer 1997/1998	0.26 ± 0.03 Spring 1997	44.6 ± 4.0 Spring 1997	22.2 ± 2.3 Spring 1998
C. crassa	3.8 ± 0.2 Autumn 1999	2.4 ± 0.3 Spring 1997	0.47 ± 0.04 Autumn 1999	0.15 ± 0.02 Spring 1997	36.6 ± 3.8 Spring 1997	17.8 ± 1.4 Autumn 1999
D. vaucheriaeformis	6.5 ± 0.4 Autumn 1997	4.6 ± 0.6 Winter 1998	0.49 ± 0.03 Summer 1997/1998	0.32 ± 0.04 Autumn 1999	41.4 ± 3.7 Autumn 1999	22.0 ± 1.8 Summer 1997/1998
G. cervicornis	5.8 ± 0.3 Autumn 1999	2.7 ± 0.2 Winter 1997	0.36 ± 0.01 Autumn 1997	0.24 ± 0.01 Winter 1998	41.5 ± 2.3 Spring 1998	20.4 ± 2.9 Winter 1997
G. tenuifrons	3.9 ± 0.3 Autumn 1998	2.2 ± 0.1 Spring 1997	0.41 ± 0.04 Summer 1997/1998	0.17 ± 0.02 Winter 1997	36.4 ± 3.1 Spring 1998	14.8 ± 1.8 Summer 1997/1998
H. valentiae	5.4 ± 0.2 Autumn 1997	4.0 ± 0.2 Winter 1998	0.56 ± 0.04 Autumn 1998	0.27 ± 0.02 Spring 1998	42.6 ± 4.0 Spring 1997	19.3 ± 0.4 Autumn 1998
R. africanum	4.5 ± 0.1 Autumn 1988	3.1 ± 0.3 Spring 1998	0.47 ± 0.04 Summer 1998/1999	0.18 ± 0.02 Spring 1998	45.1 ± 3.1 Autumn 1999	18.2 ± 1.7 Summer 1999
U. lactuca	3.6 ± 0.2 Autumn 1999	1.9 ± 0.1 Spring 1997	0.32 ± 0.03 Summer 1998/1999	0.12 ± 0.01 Spring 1998	32.4 ± 1.8 Autumn 1999	13.9 ± 1.2 Summer 1997/1998

DISCUSSION

The variation in salinity measured throughout this study (from 44.0 to 51.5 psu) shows the strong influence of the sea on the sampling site and possibly the relationships between the time of sampling and the tidal movements. Sea water (salinity ca. 35) enters through Itajuru Channel at high tide, and mixes with the high-salinity waters of the Araruama Lagoon in the area of the sampling site, accounting for lower local salinity. Inner parts of the Lagoon (Fig. 1) are weakly affected by seawater. On the other hand, at low tides the water moves from the inner parts of the Lagoon to the sea, and the water near the sampling site tend to show higher salinity. As the nine sampling events occurred in different moments of the tidal regime, variations in the salinity result from the balance between the seawater and the lagoon's water.

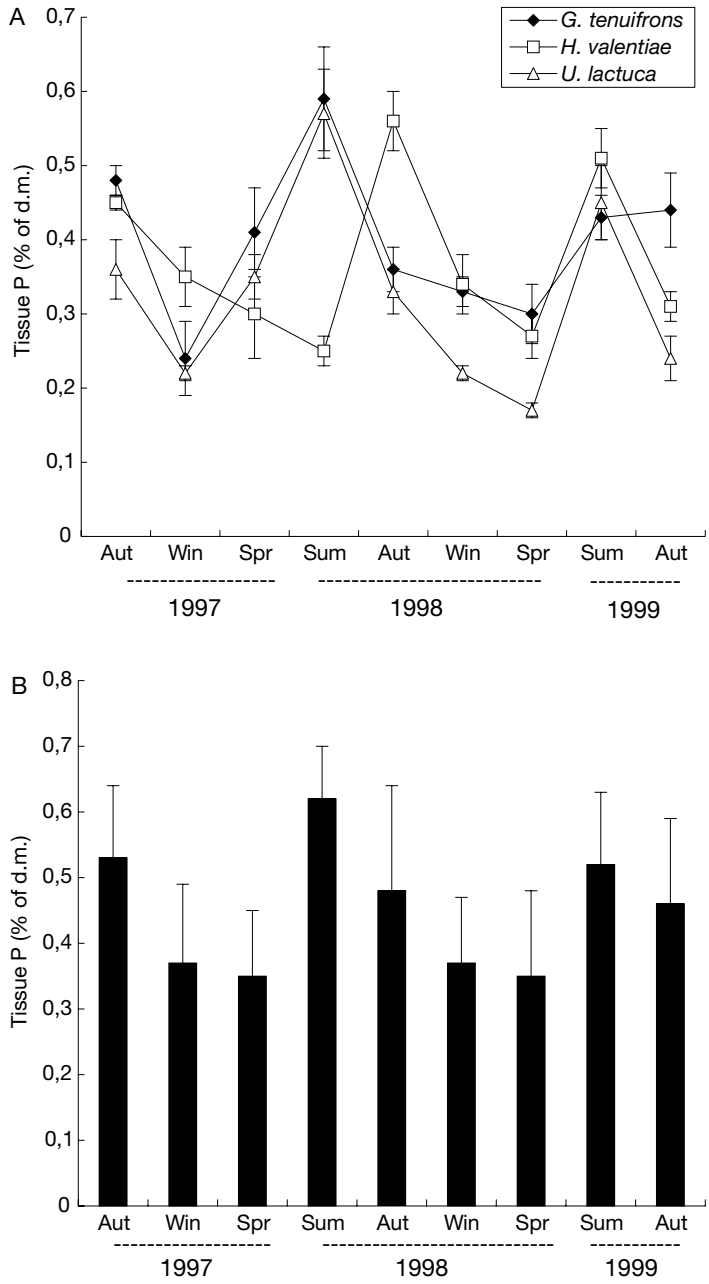

Fig. 3. Seasonal fluctuations in the content of phosphorus of *Gracilariopsis tenuifrons*, *Hypnea valentiae*, and *Ulva lactuca* (A), and mean values of P in the tissues of all macroalgae (B) collected in nine seasonal sampling in the field. Data are expressed as percentage of the dry weight (d.w.). In (A) each point represents the mean of four to six replicates ± standard deviation ($4 \leq n \leq 6$). In (B) each bar represents the mean of 32 to 44 measurements ± standard deviation ($32 \leq n \leq 44$).

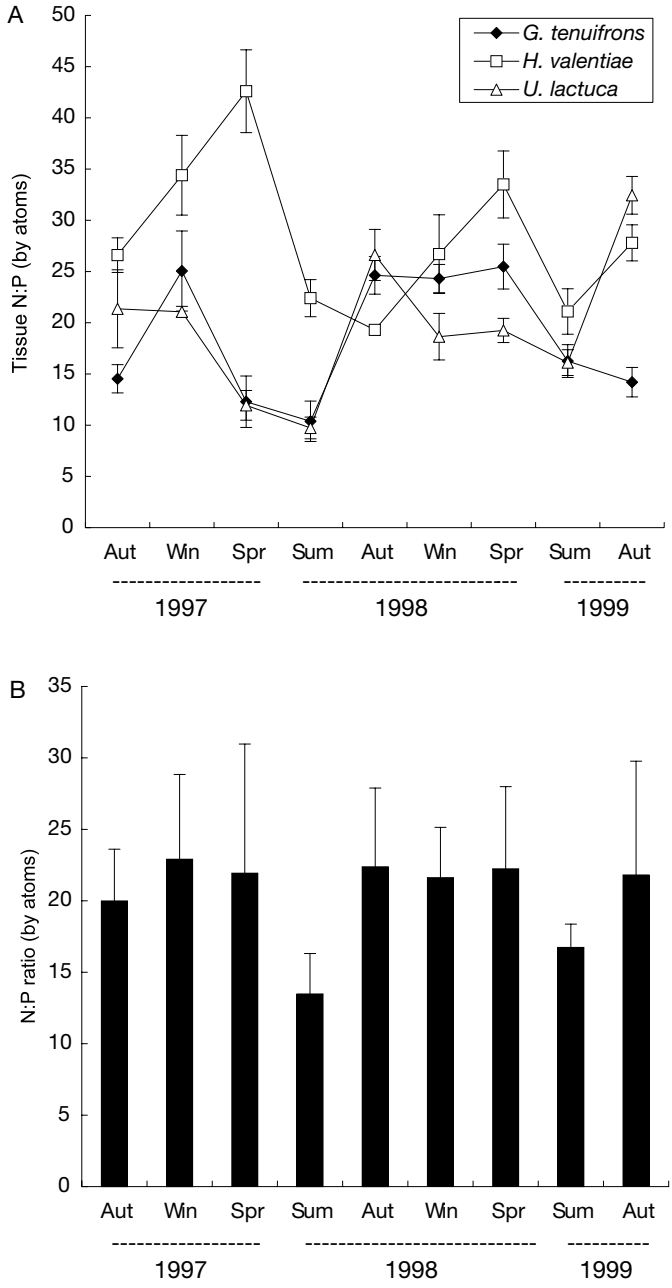

Fig. 4. Seasonal fluctuations in N:P ratio of *Gracilariopsis tenuifrons*, *Hypnea valentiae*, and *Ulva lactuca* (A), and mean values of N:P ratio in the tissues of all macroalgae (B) collected in nine seasonal sampling in the field. In (A) each point represents the mean of four to six replicates ± standard deviation ($4 \leq n \leq 6$). In (B) Each bar represents the mean of 32 to 44 measurements ± standard deviation ($32 \leq n \leq 44$).

Variations of nutrient concentration in water show that the environment may be classified as an oligotrophic to mesotrophic system, confirming the comprehensive previous study of Knoppers (1994). However, in the context of the current study results of dissolved nutrients should be considered with caution, since they only reflect the concentrations measured during the sampling of the macroalgae, and not the actual nutrient regime at the sampling site. In any case, the dissolved nutrients were detected at low to medium concentrations throughout the year, and our data show that an important enrichment of phosphorus and ammonia occurs at the sampling site during summer. An evaluation of the data on ammonia and phosphate indicate that summer represents an anomalous period, since those components attained the highest concentrations. This trend may be related to the impact of tourism in the region during part of the year, from December to March (austral summer) since most part of the local wastewater is drained to the lagoon without treatment.

The algae at the sampling site showed high contents of tissue nitrogen and phosphorus (see Wheeler & Björnsäter, 1992; Peckol *et al.*, 1994), which might be a response to high nutrient availability in the environment. As the data for dissolved nutrients show values which are predominantly low (except for ammonia and phosphate in summer), this suggests that the algae take up nutrients quickly. This seasonal input of dissolved nutrients in water in summer has an important impact on the trends of variation in tissue N, P and N:P ratio of the macroalgae, affecting the algae in summer and in the following season, autumn. Spring, when the increase in input of nutrients in water is just starting, was identified as the period of minimum levels of tissue N and P in macroalgae. Large amounts of ammonia and phosphate enter in the system during summer, and these nutrients may be taken up and assimilated by the macroalgae. Since algae show low percentages of tissue nutrients in late spring, a progressive increase of tissue nutrients during summer seems to occur. As a result the species show higher values of tissue nutrients at the end of the season (mainly for phosphorus), when the measurements were performed.

In the case of hypersaline systems, the water turnover time is comparatively long, which in turn leads to deteriorating water quality in response to even modest pollution loading. Araruama Lagoon, connected to the coastal ocean via a single channel, shows a flushing half-life that measures 83.5 days, considerably longer than for other coastal lagoons (Kjerfve *et al.*, 1996). In other words, inputs of pollution may affect the organisms for long periods, since the water exchange is extremely slow in Ararauama lagoon.

In environments such as Araruama Lagoon, primary production tends to be limited due the control of phosphorus by reactions with calcium carbonate, especially at the sediment-water interface (Knoppers *et al.*, 1996). In contrast, nitrogen may be abundant. The components of the microalgal mats are predominantly cyanobacteria (Baeta Neves, 1983), organisms that present high tolerance to environmental variations. This may explain the competitive advantage of the microphytobenthos over the macroscopic algae of the Lagoon as primary producers, preventing macroalgal blooms such as in other coastal systems (Valiela *et al.*, 1997; Pihl *et al.*, 1999; Naldi & Viaroli, 2002). As a consequence, a proliferation of seaweeds would be unlikely to happen due to competition with microphytobenthic species.

Except for *U. lactuca* in three measurements, all macroalgae showed values for tissue nitrogen higher than 2% of d.w., the critical N level for maximum growth according to Hanisak (1979). These finding may suggest that, even considering the competition for nitrogen with microphytobenthos and phytoplankton,

the nitrogen availability is enough to provide algae with high concentrations of this element. Thus, considering N content, all species would be in good condition for growth throughout most of the year. As the plants do not grow constantly during the year (Lavrado *et al.*, unpublished data) other factors may play a key role in the control of growth. Despite the climatic stability of tropical environments, higher temperatures and excessive light in summer seem to play an important role in triggering a biomass decline during hot months in Araruama Lagoon. As a consequence, decomposition of algal tissues is common in the Lagoon, such as demonstrated by Menéndez *et al.* (2001) in a shallow Mediterranean coastal lagoon in the summer. Another hypothesis may be related to the actual critical level of nitrogen to the macroalgae in the Brazilian tropical environments. In a directly related study, Lourenço *et al.* (unpublished data) found that the mean values for 50 species of macroalgae from oligotrophic sites and an upwelling area are always higher than 2% d.w. (including data measured in calcareous algae in the final mean values for the environments) over the year, with few exceptions.

The study performed by Atkinson & Smith (1983) gives important data on the C:N:P composition of benthic plants over the world, but it does not show values for concentration of N and P in the tissue (only the atomic ratios). In addition, there is no information about the season in which each sampling was performed, as well as the maximum and minimum values recorded, making it difficult to compare results. Regarding our data for N:P ratio, there is a relative disagreement with those authors; our highest results for *A. spicifera* and *U. lactuca* represent similar (but lower, in several observations) measurements to those reported by Atkinson & Smith (1983) for *A. spicifera* (N:P = 38:1; from Hawaii) and *Ulva* sp. (N:P = 35:1; from Rhode Island); other results show greater disagreements. On the other hand, our data for N:P ratio can easily be compared with data from studies that measure seasonal fluctuations in N:P ratio. This assessment indicates that our data for N:P ratio may vary in a similar way if compared with data determined for species from temperate and subtropical environments, despite differences in the species composition. For instance, we found, for *U. lactuca*, similar seasonal ranges of variation to those presented by Wheeler & Björnsäter (1992) for common species of Oregon coastal waters (*Enteromorpha intestinalis*, *Ulva fenestrata*, from 10.1:1 to 30.1:1), Peckol *et al.* (1994) for the green alga *Cladophora vagabunda* (from 20:1 to 36:1), and Flores-Moya *et al.* (1995) for the brown alga *Phyllariopsis purpurascens* (from 14:1 to 40:1). However, our results were lower than the mean values of tissue N:P presented by Sfriso & Marcomini (1999) for *Ulva rigida* in two different sites of Venice Lagoon (50:1 and 72:1, respectively). The divergences among results are probably related to phosphorus determinations rather than N analysis. Low stability of phosphorus in acid medium after digestion may lead to underestimation of P content, and as a consequence establish higher N:P ratios. In order to prevent this analytical problem, chemical reaction should be performed as soon as possible after acid digestion.

According to the Björnsäter & Wheeler (1990) classification of nutrient status of macroalgae based on N:P ratio of tissues, a N:P ratio < 16 indicates N-limitation; a N:P ratio 16 – 24 indicates N-sufficiency and P-sufficiency – i.e. no limitation, and a N:P > 24 indicates P-limitation. Applying this classification to our data we could conclude that the macroalgal community in the sampling site is permanently N-sufficient, with few exceptions. However, this classification must be considered with caution, because the ranges may not be suitable for macroalgae from tropical environments such as Araruama Lagoon. Further investigations are needed to test the suitability of that classification for tropical environments.

The macroalgae are apparently almost permanently P-deficient, except in summer when the levels of tissue P attain the highest concentrations, probably related to the input of domestic wastewater, which includes a great amount of phosphorus-containing substances such as detergents. On the other hand, the turnover of P is very high, and there may be a continuous availability of P to the algae, which may be quickly taken up. Data of N:P ratios should be evaluated with care, since raw measurements of tissue N and P must also be interpreted. For example, the mean N:P ratio measured in summer was lower than 16:1 in 1998 (13.5:1), and near 16:1 in 1999 (16.7:1) (Fig. 3C). The mean value for tissue N was similar in the two seasons (3.7 and 3.9 of the d.w., respectively), and the variations in tissue N:P were affected mainly by the differences in tissue P. The overall mean values for tissue nitrogen and phosphorus in all algae collected of the Lagoon during the nine sampling are 3.88 ± 1.27 and 0.31 ± 0.10 % of d.w. (n = 285), respectively. This mean value for tissue P is not actually a low level of phosphorus, and it is higher than many other algae / study areas elsewhere (See Wheeler & Björnsäter 1992; Peckol *et al.* 1994). The high mean overall N:P ratio observed in Araruama Lagoon (29.3 ± 9.3, n = 285) is strongly affected by the elevated concentrations of nitrogen.

Macroalgae live in special conditions in the Lagoon. The lack of hard substrates selects for species capable of floating permanently (e.g. *U. lactuca*), being partially buried in sediment (e.g. *C. crassa*, *G. cervicornis* and *G. tenuifrons*) or to tumbling on the bottom under water movement (e.g. *H. valentiae* and *R. africanum*). Except for *C. crassa* all these species may be found in large biomass in (at least) part of the year, and it may indicate high adaptation to those special ways of life. Relatively few of the abundant macroalgal species found in southeast Brazil are capable of living under these unfavourable conditions. In addition, the high variation in salinity constitutes an extremely selective factor. However, part of the Lagoon's species lives in conditions similar to those found in the sea, such as *A. spicifera* and *D. vaucheriaeformis*. These species are not found in large biomass, but they are always attached to rocks, almost restricted to the Itajuru Channel area, the part of the Lagoon that receives maximum influence of the sea.

The finding that in autumn algae shows the highest contents of tissue N and the second peak of tissue P agrees with the results of Knoppers *et al.* (1996) on the Lagoon's primary production. Those authors found that in autumn benthic primary production is two-fold higher than in spring. Our data, indicating high contents of tissue N and P in macroalgae in autumn, suggest the possibility of higher macroalgal primary productivity in that season. We hypothesise that primary producers are drastically affected by excessive light and high temperatures in part of the spring and in the summer. This could initiate losses of tissue and nutrients to the environment due to the decomposition process (Hanisak, 1993; Menéndez *et al.*, 2001). In autumn, temperature and light do not seem to cause a strong disturbance to the algae. It is possible that phosphorus is available in the water in high concentrations during early autumn (due the summer input) and may provide favourable conditions for growth. This means that the nutrient enrichment recorded in summer affects the chemical characteristics of the macroalgae in autumn.

Environmental variations are reported as small in tropical systems. Our results indicate that, despite the climatic stability in the environment studied, large fluctuations in N, P and N:P ratio occur in the macroalgae, comparable to those reported to temperate regions. These changes seem to be linked to the nutrient input, as well as the fluctuations in the high levels of salinity. In conclusion, we rejected our initial hypothesis and demonstrated that significant fluctuations in

tissue N and P occur in the seaweeds of the site studied. These fluctuations are comparable in magnitude to those reported for seaweeds of temperate environments, although they are generated by different factors (e.g. nutrient and temperature in temperate environments, anthropogenic loads of nutrients and salinity in Araruama Lagoon). We are currently evaluating the tissue N and P composition of many other marine species of Brazil. New results will be available soon and could contribute to the understanding of the nutrient metabolism of tropical seaweeds.

Acknowledgements. We are indebted to FAPERJ (Foundation for Research Support of Rio de Janeiro State, grant E-26.170.283/97) for the financial support to this study. A special acknowledgement is due to Dr Yocie Yoneshigue-Valentin (Universidade Federal do Rio de Janeiro) for offering us laboratory facilities, field support for performing this study, and for the identification of part of the samples. We also acknowledge Dr Maria A. Esquibel, Dr Celso Lage and Dr Anaize B. Henriques for offering us laboratory facilities. The authors are grateful to MSc André Taouil, BSc Daniela Gabriel, MSc Lisia M.S. Gestinari, MSc Isolda C. Bravin, Dr Carlos J. David, BSc José Carlos F. Gomes Filho, BSc Gerson N. Oliveira, MSc Joel C. De-Paula, and MSc Valéria Cassano their assistance in the field. We also thank L.M.S.G., J.C.De-P. and V.C. for their assistance in the identification of the species. Thanks are due to Dr Roberto C. Villaça (Universidade Federal Fluminense) for his French translation of the abstract. S.O.L. acknowledges CNPq (National Council for the Development of Science and Technology) that provided him individual research fellowships (grants 301.636/96-6 and 350.033/02-1).

REFERENCES

ATKINSON M.J. & SMITH S.V., 1983 — C:N:P ratios of benthic marine plants. *Limnology and Oceanography* 28: 568-574.

BAETA NEVES M.H.C., 1983 — Flora ficológica da lagoa hipersalina de Araruama (Estado do Rio de Janeiro – Brasil) 2. Cyanophyceae. *Instituto de Pesquisas da Marinha* 149: 1-18.

BJÖRNSÄTER B.R. & WHEELER P.A., 1990 — Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). *Journal of Phycology* 26: 603-611.

DUKE C.S., LITAKER W. & RAMUS J. 1989 — Effect of temperature on nitrogen-limited growth rate and chemical composition of *Ulva curvata* (Ulvales: Chlorophyta). *Marine Biology* 100:143-150.

FLORES-MOYA A., FERNÁNDEZ J.A. & NIELL F.X., 1995 — Seasonal variations of photosynthetic pigments, total C, N, and P content, and photosynthesis in *Phyllariopsis purpurascens* (Phaeophyta) from the Strait of Gibraltar. *Journal of Phycology* 31: 867-874.

FONG P., BOYER K.E. & ZEDLER J.B., 1998 — Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L.) Link. *Journal of Experimental Marine Biology and Ecology* 231: 63-79.

FONG P., FOIN T.C., ZEDLER J.B. & DONOHOE R.M., 1994 — A simulation model of lagoon algal communities based on competition for nitrogen and internal storage. *Ecological Monographs* 64: 225-247.

FONG P., KAMER K., BOYER K.E. & BOYLE K.A., 2001 — Nutrient content of macroalgae with different morphologies may indicate sources of nutrients for tropical marine systems. *Marine Ecology Progress Series* 220: 137-52.

FONG P., ZEDLER J.B. & DONOHOE R.M., 1993 — Nitrogen vs. Phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology and Oceanography* 38: 906-923.

FUJITA R.M., 1985 — The role of nitrogen status in regulation transient ammonium uptake and nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology* 92: 283-301.

GRASSHOFF K., EHRHARDT M. & KREMLING K., 1983 — *Methods of Seawater Analysis*. 2nd revised and extended ed. Weinheim, Verlag Chemie, 419 p.

HACH C.C., BOWDEN B.K., KOPELOVE A.B. & BRAYTON S.T., 1987 — More powerful peroxide Kjeldahl digestion method. *Journal of the Association of Official Analytical Chemists* 70: 783-787.

HANISAK M.D., 1979 — Nitrogen limitation of *Codium fragile* ssp. *tomentosoides* as determined by tissue analysis. *Marine Biology* 50: 333-337.

HANISAK M.D., 1993 — Nitrogen release from decomposing seaweeds: species and temperature effects. *Journal of Applied Phycology* 5:175-181.

KJERFVE B., SCHETTINI C.A.F., KNOPPERS B.A., LESSA G. & FERREIRA H.O., 1996 — Hydrology and salt balance in a large, hypersaline coastal lagoon: Lagoa de Araruama, Brazil. *Estuarine, Coastal and Shelf Science* 42: 701-725.

KNOPPERS B. 1994 — Aquatic primary production in coastal lagoons. *In:* Kjerfve, B. (ed.) *Coastal lagoon process*. Amsterdam, Elsevier, pp. 243-286.

KNOPPERS B., DE SOUZA W.F.L., DE SOUZA M.F.L., GONZALEZ RODRIGUEZ E., VIANA E.F. DA C. & VIANNA A.R., 1996 — *In situ* measurements of benthic primary production, respiration and nutrient fluxes in a hypersaline coastal lagoon of SE Brazil. *Revista Brasileira de Oceanografia* 44: 153-163.

LAPOINTE B.E. & DUKE C.S., 1984 — Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *Journal of Phycology* 20: 488-495.

MENÉNDEZ M., MARTÍNEZ M. & COMÍN F.A., 2001 — A comparative study of the effect of pH and inorganic carbon resources on the photosynthesis of three floating macroalgae species of a Mediterranean coastal lagoon. *Journal of Experimental Marine Biology and Ecology* 256: 123-136.

NALDI M. & VIAROLI P., 2002 — Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agard in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). *Journal of Experimental Marine Biology and Ecology* 269: 65-83.

PARSONS T.R., MAITA Y. & LALLI C.M., 1984 — *A manual of chemical and biological methods for seawater analysis*. Oxford, Pergamon Press, 173 p.

PECKOL P., DEMEO-ANDERSEN B., RIVERS J., VALIELA I., MALDONADO M. & YATES J., 1994 — Growth, nutrient uptake capacities and tissue constituents of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loading rates. *Marine Biology* 121: 175-185.

PIHL L., SVENSON A., MOKSNES P.-O. & WENNHAGE H., 1999 — distribution of green algal mats throughout shallow soft bottoms of the Swedish Skagerrak archipelago in relation to nutrient sources and wave exposure. *Journal of Sea Research* 41: 281-294.

REIS R.P. & YONESHIGHE-VALENTIN Y., 1996 — Distribuição das macroalgas na Lagoa de Araruama, estado do Rio de Janeiro, Brasil. *Revista Brasileira de Botânica, São Paulo* 19: 77-85.

RIVERS J.S. & PECKOL P., 1995 — Interactive effects of nitrogen and dissolved inorganic carbon on photosynthesis, growth, and ammonium uptake of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae*. *Marine Biology* 121: 747-753.

SFRISO A. & MARCOMINI A., 1999 — Macrophyte production in a shallow coastal lagoon. Part II. Coupling with sediment, SPM and tissue carbon, nitrogen and phosphorus concentration. *Marine Environmental Research* 47:285-309.

SCHAFFELKE B. 1999 — Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. *Marine Ecology Progress Series* 182:305-310.

VALIELA I., FOREMAN K., TEAL J.M., HOWES B. & AUBREY D., 1990 — Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. *Biogeochemistry* 8: 177-197.

VALIELA I., McCLELLAND J., HAUXWELL J., BEHR P.J. & FOREMAN K., 1997 — Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105-18.

WATKINS K.L., VEUM T.L. & KRAUSE G.F., 1987 — Total nitrogen determination of various sample types: a comparison of Hach, Kjeltec and Kjeldahl methods. *Journal of the Association of Official Analytical Chemists* 70: 410-412.

WHEELER P.A. & BJÖRNSÄTER B.R., 1992 — Seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific northwest coast. *Journal of Phycology* 28: 1-6.

WYNNE M.J., 1998 — A checklist of benthic marine algae of the tropical and subtropical western Atlantic: first revision. *Nova Hedwigia* 116:1-155.

ZAR J.H., 1996 — *Biostatistical Analysis*. Upper Saddle River, NJ, Prentice Hall, Inc., 920 p.