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Tissue nitrogen and phosphorus in seaweeds in a tropical eutrophic environment: What a long-term study tells us

Sergio O. Lourenço,* Elisabete Barbarino, Andyara Nascimento, Joana N.P. Freitas & Graciela S. Diniz
Departamento de Biologia Marinha, Universidade Federal Fluminense, Caixa Postal 100644, CEP 24001-970, Niterói, RJ, Brazil

*Author for correspondence: e-mail solourenco@yahoo.com; fax: +55 21 2629 2292

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Abstract

Percentages of nitrogen and phosphorus in 10 species of seaweeds (6 green and 4 red algae) were monitored from 1997 to 2004 by seasonal sampling in Guanabara Bay, South-eastern Brazil. The species did not show consistent variations in tissue *N*, *P* and *N:P* that related to annual cycles. Throughout this study, higher percentages of tissue *N* and *P* were found in *Bostrychia radicans* and *Grateloupia doryphora* (red algae) and lower in *Cladophora rupestris* and *Codium decorticatum* (green algae). In November 1999, the Icaraí Submarine Sewage Outfall became operational, resulting in a reduction of visual pollution in the area and an improvement in the local quality of seawater for recreational use. Measurements of dissolved nutrients at the sampling site did not indicate significant changes in concentrations after the commissioning of the submarine sewage outfall; however, tissue *P* and *N:P* ratio of most of species were significantly lower than in the first two years of this survey. Variations in tissue nitrogen throughout this study were not significant, except for *G. doryphora* in some comparisons. Results show that seaweeds function very well as monitors of environmental changes in Guanabara Bay. Experimental data are needed to identify possible environmental processes which are promoting changes in chemical composition of the local seaweed populations.

Introduction

Anthropogenic inputs of nutrients have had remarkable impacts on marine organisms in coastal areas (Clark, 2001). Increased abundance of opportunistic seaweeds is among the general consequences of nutrient loading in coastal areas (Rivers & Peckol, 1995). Macroalgae respond to nutrient enrichment by taking up nutrients, growing, and storing "excess" nutrients for future growth (Fujita, 1985; Björnsäter & Wheeler, 1990). The proliferation of opportunistic seaweeds affects local biodiversity and may promote a decrease in concentrations of dissolved nutrients in the water column (Rivers & Peckol, 1995; Valiela et al., 1997).

Concentrations of tissue nutrients reflect the environmental conditions of the site, providing a useful indicator of local nutrient status (Fong et al., 1994). In addition, total nutrient concentration in the algal tissue provides an integrated measurement of nutrient regime over time (Wheeler & Björnsäter, 1992; Villares & Carballeira, 2003). Monitoring of tissue nutrients to detect enrichment can be undertaken at less frequent intervals than monitoring of the water-column nutrients, and allows a more accurate evaluation of the nutrient status of the macroalgae. Studies on tissue *N* and *P* content of macroalgae predominantly in temperate coastal environments (Wheeler & Björnsäter, 1992; Peckol et al., 1994) reveal wide fluctuations in the tissue content of *N* and *P* related to seasonal changes and nutrient availability. By comparison, information on tissue *N* and *P* of algae from tropical environments is relatively scarce (Schaffelke, 1999; Fong et al., 2001), and more data are needed from those regions.

Guanabara Bay, Brazil, is a eutrophic coastal environment connected to the sea by a narrow mouth, which partially restricts water exchange. The Bay receives substantial river runoff relative to the total water volume, making it similar to a large estuary in its inner parts (Kjerfve et al., 1997). The Bay is located in a very populated urban area, and long-term cultural eutrophication has generated an environment with permanent high concentrations of dissolved nutrients due to output of both domestic and industrial wastewater (Mayr et al., 1989). Considering these characteristics, we hypothesised that the seaweeds of Guanabara Bay would present permanently high concentrations of tissue *N* and *P* and show no significant variations in their tissue nutrients throughout the year and no inter-annual changes in tissue nutrients.

In this study we report on the seasonal variations of tissue *N*, *P* and *N*:*P* atomic ratio of ten abundant macroalgal species of Guanabara Bay. Comparisons were made between algal *N* and *P* contents and the concentrations of dissolved nutrients in the system in this 7-year assessment. In addition, during this study a submarine sewage outfall was built in the study area and its possible effects on the tissue composition of the macroalgal flora was evaluated.

Materials and methods

Study area

The sampling site is located at Boa Viagem Beach (23°04′S, 43°08′W), in Guanabara Bay. The site is in the urban area of Niterói City, and it is located near the entrance of the Bay (Figure 1), which promotes a local dilution in the typical high levels of pollution of the Bay. The Bay shows a low water exchange rate (Mayr et al., 1989) due to geomorphological features and human occupation of coastal areas. Guanabara Bay comprises an area of 381 km^2 and an estimated 2 billion m^3 of water. The catchment area (4000 km^2) includes 35 rivers that contribute substantially to the freshwater input. The mean depth is 7.7 m, varying from 50 m (main channel) to less than 1 m in the inners parts close to the internal margins. The area of Guanabara Bay comprises 15 municipalities, with a population of ca. 7.6-milion inhabitants (FEEMA, 1999). According to Paranhos et al. (2001), ca. 470 t of BOD and 150 t of industrial sewage are disposed of daily into the Bay. CIBG (2004) indicates that in 1994 ca. 8 t of oil derivatives and 55 kg of heavy metals were disposed of daily into the Bay by the 55 main industrial plants in the area. An ongoing program has been reducing many sources of pollution into the Bay, but the total amount of pollutants that enters the system daily is still high (CIBG, 2004).

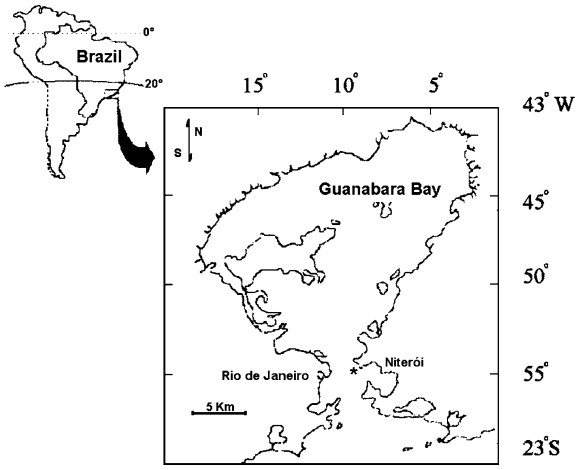

Figure 1. Sampling site in Guanabara Bay. * = Sampling site.

Taouil and Yoneshigue-Valentin (2002) classified the sampling site as moderately affected by wave action, with an unusual abundance of pebbles and large-grain sand in the intertidal area.

Algae studied

In this study ten macroalgal species were analysed. The identification of the macroalgae was carried out following the checklist of Wynne (1998). Experts were consulted to confirm our identifications.

Chlorophyta: *Chaetomorpha antennina* (Bory) Kützing, *Cladophora rupestris* (L.) Kützing, *Codium decorticatum* (Woodw.) M. Howe, *Enteromorpha flexuosa* (Wulfen) J. Agardh, *Ulva fasciata* Delile, and *Ulva lactuca L.*

Rhodophyta: *Bostrychia radicans* (Mont.) in Orbigny, *Chondracanthus teedii* (Mertens ex Roth) Fredericq, *Grateloupia doryphora* (Montagne) M. Howe, and *Gymnogongrus griffthsiae* (Turner) Mart.

The species are found attached to the rocks and pebbles of the sampling site (water column between 0.4 to 0.8 m). Samples of two species (*B. radicans* and *C. anteninna*) were collected at Itapuca Stone, a site located 400 m from Boa Viagem Beach, where they were more abundant (attached to vertical rock surfaces). We assume that both sampling sites have virtually the same environmental characteristics (temperature,

salinity, pollution, dissolved nutrients, etc. – data not shown), except for more water movement at Itapuca Stone.

Sampling

Sampling began in June 1997 (austral autumn) and continued through June 2004. Samples were collected seasonally for a total of 29 field trips, and each sampling occurred in the last 3 weeks of each season.

Whole thalli of adult plants were collected in the early morning and washed in the field with seawater to remove epiphytes, sediment and detritus. At least 15 whole plants of each species were collected, independent of the size of each seaweed. All species were typically found at the same specific points in the site throughout the study (e.g. *C. antennina* was sampled always at the same rocks near the Itapuca Stone; *C. decorticatum* was found always attached to the pebbles near the Boa Viagem Island). The plants were placed 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 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 through cellulose membrane filters (Millipore® HAWP 0.45 μm pore) and kept at −20 °C for spectrophotometric determinations of ammonium, nitrate, and nitrite (Parsons et al., 1984), phosphate and urea (Grasshoff et al., 1983). Each sample was measured at least three times to obtain accurate results, and the results showed in this study represent mean values for four independent samples collected in the field for each sampling.

Tissue analysis

Total *N* and *P* were determined in algal tissue after peroxymonosulphuric acid digestion, using a Hach digestor (Digesdhal®, Hach Co.) (Hach et al., 1987). Samples containing 50 to 200 mg (dry matter) were digested with 4 ml concentrated sulphuric acid (Merck Co.) at 440 °C and treated with 17 ml of 30% hydrogen peroxide (Merck Co.). Total nitrogen and phosphorus contents in the samples were determined spectrophotometrically after specific chemical reactions. See Lourenço et al. (2005) for analytical details.

For each species and sampling at least four to six independent (from different plants) measurements of tissue *N* and *P* were performed ($4 \leq n \leq 6$).

Statistical analysis

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

Results

Nitrite and urea showed the lowest concentrations among dissolved *N* sources, typically lower than 3.0 μM. Ammonium and nitrate showed higher concentrations, varying in most of the observations between 5.0 and 15.0 μM. Variations of phosphate concentrations fluctuated between 0.4 and 2.6 μM. Wide variations in total dissolved nitrogen and *N*:*P* ratio were recorded, but no seasonal trend was detected throughout the study. Salinity fluctuated around 31 psu throughout the survey (Table 1).

Small variations in tissue nitrogen were recorded in the species, with high values (>5.0%) throughout the study. This general description is exemplified by *C. decorticatum*, *G. doryphora* and *U. fasciata* (Figure 2A), visually the most abundant species in the sampling site. Among all species, *Bostrychia radicans* and *Grateloupia doryphora* (red algae) exhibited the highest values for tissue *N* and *Chaetomorpha antennina*, *Cladophora rupestris* and *Codium decorticatum* the lowest (Table 2). The tissue *N* content of all species were not significantly different for the vast majority of the paired comparisons tested (ANOVA, $F_{28,907} = 3.289$) (Figure 2B); some comparisons involving data of 1997 (higher values) and 2003–2004 (lower values) exhibited significant differences.

Values for tissue *P* showed wider variations among the species than the values of tissue *N*. Most of the comparisons showed significant differences, with a consistent trend of higher values for tissue *P* in 1997–1999 and lower values in 2000–2004 (Figure 3A, B).

Table 1. Measurements of salinity and dissolved nutrients at the sampling site in Guanabara Bay. Data represent the mean ± standard deviation ($n = 4$) and are expressed as μM, except for salinity (psu).

Sampling	*N*-ammonium	*N*-nitrite	*N*-nitrate	*N*-Urea	*P*-phosphate	Total dissolved *N*	*N:P* Ratio	Salinity
Autumn 1997	12.9 ± 4.67	1.46 ± 0.53	0.35 ± 0.07	0.18 ± 0.04	0.96 ± 0.31	14.9 ± 4.80	15.5 ± 4.59	34.8
Winter 1997	7.30 ± 0.78	1.73 ± 0.15	0.85 ± 0.21	0.31 ± 0.05	1.39 ± 0.02	10.2 ± 0.73	7.30 ± 0.50	32.0
Spring 1997	13.2 ± 2.61	1.77 ± 0.09	2.72 ± 0.46	0.41 ± 0.08	1.97 ± 0.09	18.1 ± 3.01	9.19 ± 1.24	32.0
Summer 1998	16.0 ± 2.26	1.06 ± 0.22	1.69 ± 0.30	0.39 ± 0.08	2.04 ± 0.21	19.4 ± 2.44	9.51 ± 2.08	31.3
Autumn 1998	11.6 ± 4.34	2.32 ± 0.57	3.59 ± 1.78	1.10 ± 0.21	1.26 ± 0.47	18.6 ± 2.64	14.8 ± 5.49	29.8
Winter 1998	6.81 ± 1.93	1.87 ± 0.22	5.48 ± 0.97	1.20 ± 0.40	1.38 ± 0.42	15.4 ± 2.15	11.1 ± 4.02	31.0
Spring 1998	5.25 ± 2.29	1.33 ± 0.81	2.55 ± 1.72	0.82 ± 0.12	2.62 ± 1.57	9.95 ± 3.40	3.80 ± 1.72	32.5
Summer 1999	4.10 ± 2.06	1.18 ± 0.40	1.53 ± 0.92	0.38 ± 0.20	1.49 ± 0.33	7.19 ± 1.56	4.83 ± 2.19	28.5
Autumn 1999	10.8 ± 3.99	3.87 ± 1.57	1.24 ± 0.45	1.02 ± 0.21	1.81 ± 0.49	16.9 ± 5.08	9.35 ± 3.39	31.9
Winter 1999	7.84 ± 2.23	1.33 ± 0.15	3.54 ± 0.62	2.81 ± 0.36	1.23 ± 0.20	15.5 ± 2.51	12.6 ± 4.60	31.8
Spring 1999	4.63 ± 1.15	0.78 ± 0.23	1.44 ± 0.56	2.49 ± 0.43	2.64 ± 1.22	9.34 ± 2.30	3.54 ± 1.54	30.1
Summer 2000	3.53 ± 0.96	1.12 ± 0.51	2.63 ± 1.35	3.22 ± 0.24	1.84 ± 0.34	10.5 ± 2.76	5.71 ± 0.43	32.6
Autumn 2000	9.05 ± 1.58	1.10 ± 0.58	11.0 ± 1.27	1.42 ± 0.23	1.70 ± 0.37	22.6 ± 12.8	13.3 ± 2.07	30.8
Winter 2000	16.0 ± 1.10	2.03 ± 0.38	9.80 ± 2.57	1.59 ± 0.35	2.05 ± 0.30	29.4 ± 3.32	14.4 ± 2.35	33.4
Spring 2000	11.9 ± 3.95	0.78 ± 0.11	4.62 ± 0.43	1.22 ± 0.24	2.37 ± 0.42	18.5 ± 3.98	7.81 ± 2.23	32.7
Summer 2001	2.27 ± 0.67	0.36 ± 0.07	2.39 ± 0.89	1.36 ± 0.26	0.87 ± 0.47	6.38 ± 1.23	7.33 ± 4.69	29.9
Autumn 2001	2.40 ± 1.89	1.03 ± 0.18	9.77 ± 3.42	3.45 ± 0.76	0.43 ± 0.05	16.7 ± 3.17	38.7 ± 5.10	31.7
Winter 2001	12.3 ± 1.14	1.15 ± 0.16	7.47 ± 1.79	3.59 ± 0.87	1.26 ± 0.17	24.5 ± 0.60	19.5 ± 3.5	32.0
Spring 2001	7.41 ± 1.11	1.68 ± 0.07	9.28 ± 0.43	3.69 ± 0.32	1.89 ± 0.11	22.1 ± 1.94	11.7 ± 1.6	31.4
Summer 2002	3.90 ± 1.93	1.08 ± 0.41	6.12 ± 1.68	3.03 ± 0.35	1.41 ± 0.14	14.1 ± 2.25	10.0 ± 1.8	32.5
Autumn 2002	6.01 ± 3.68	1.24 ± 0.41	6.33 ± 4.14	5.80 ± 0.89	1.84 ± 0.80	19.4 ± 5.90	10.5 ± 5.9	33.4
Winter 2002	5.36 ± 2.68	1.73 ± 0.37	10.7 ± 3.55	1.79 ± 0.13	1.76 ± 0.33	19.6 ± 6.50	11.1 ± 1.43	33.8
Spring 2002	5.03 ± 1.53	0.73 ± 0.24	6.71 ± 1.52	1.54 ± 0.20	1.38 ± 0.42	14.0 ± 3.04	10.2 ± 2.36	31.6
Summer 2003	8.83 ± 0.77	0.88 ± 0.11	3.15 ± 0.47	3.27 ± 0.92	1.54 ± 0.08	16.1 ± 2.53	10.5 ± 1.44	33.3
Autumn 2003	19.6 ± 1.08	1.93 ± 0.11	5.97 ± 1.21	1.54 ± 0.45	1.50 ± 0.13	29.0 ± 1.37	19.4 ± 2.12	33.9
Winter 2003	16.0 ± 1.67	2.99 ± 0.04	6.60 ± 1.18	1.34 ± 0.15	1.59 ± 0.07	28.5 ± 1.31	17.9 ± 1.4	34.4
Spring 2003	12.1 ± 2.15	2.39 ± 0.39	7.42 ± 1.96	1.68 ± 0.30	1.86 ± 0.47	23.6 ± 1.86	12.7 ± 3.5	31.5
Summer 2004	11.7 ± 1.38	2.37 ± 0.17	11.2 ± 1.65	1.53 ± 0.27	1.37 ± 0.21	26.8 ± 3.03	19.6 ± 3.3	27.1
Autumn 2004	15.0 ± 4.24	3.64 ± 1.01	7.88 ± 3.79	1.35 ± 0.41	1.92 ± 0.67	27.9 ± 9.26	14.5 ± 1.07	30.5

Among all species *Chondracanthus teedii* and *Enteromorpha flexuosa* exhibited the highest values for tissue *P* and *Cladophora rupestris* and *Codium decorticatum* the lowest (Table 2). Overall trends of *P* concentrations in tissues of all species and in the three dominant species are the same, with a significant decrease in the values in the last three years (ANOVA, $5.306 \leq F_{28,907} \leq 14.993$, $0.05 < p \leq 0.001$) (Figure 3A and B, Table 2).

Tissue *N:P* ratio was predominantly >20:1 throughout the study. In the first 30 months of the study the average (*N:P*) ratio was ca. 23:1, increasing to ca. 28:1 from summer 2001 until the end of the survey (Figure 4A, B). Similarly, the combined (*N:P*) analysis indicated significantly different between these two contrasting periods (ANOVA, $5.242 \leq F_{28,907} \leq 10.530$, $0.05 < p \leq 0.001$). *Ulva lactuca* presented the highest (*N:P*) ratio among all species, in spring 2001 (56.8:1), and *Chondracanthus teedii* the lowest, in spring 1998 (13.6:1) (Table 2).

In December 1999 the Icaraí Submarine Sewage Outfall became operational. Since then, most of the local sewage receives secondary treatment and it is released ca. 2.5 km from the sampling site, and close (ca. 1.0 km) to the entrance of Guanabara Bay. Despite the fact that there was no difference in the concentrations of dissolved nutrients recorded in the sampling site during the study (Table 1), the initiation of this facility coincides with the main divergence among sets of results for tissue *P* and (*N:P*) ratio.

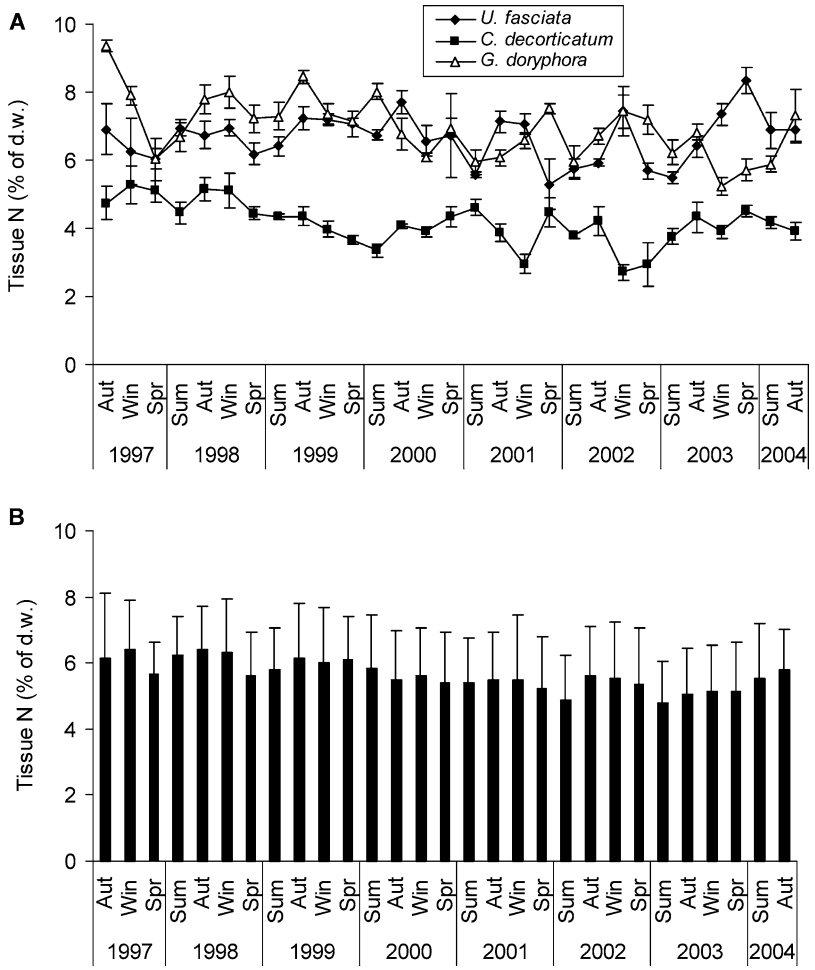

Figure 2. Seasonal fluctuations in the content of nitrogen in *Codium decorticatum*, *Grateloupia doryphora*, and *Ulva fasciata* (A), and mean values of *N* in the tissues of all macroalgae (B) collected in 29 seasonal samplings in Guanabara Bay. Data are expressed as percentage of the dry weight (d.w.). 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 112 to 144 measurements ± standard deviation ($112 \leq n \leq 1.44$).

Discussion

Dissolved nutrients were detected at medium to high concentrations throughout this study and our data show that no seasonal enrichment of nutrients occurred at the sampling site (e.g. upwelling events, seasonal increase of the volume of sewage, etc.). The high values of ammonium and urea are consistent with the discharge of large volume of domestic sewage in the area (Lavrado et al., 1991; Paranhos et al., 1997). This interpretation is supported by studies by other authors, who confirm the large amount of domestic sewage released into the Bay (Paranhos et al., 1995; FEEMA, 1999). The seaweeds at the sampling site showed high contents of tissue nitrogen and phosphorus by comparison with other studies done in tropical environments (e.g. Fong et al., 2003; Hwang et al., 2004) and similar to results obtained with seaweeds growing in an excess of nutrients (Lapointe et al., 2004).

In the case of partially closed systems, the water turnover rate is comparatively low, which in turn leads to deteriorating water quality in response to even modest pollution loading. Guanabara Bay is connected to the coastal ocean via a 4-km mouth, and has a flushing half-life of 6.5 d, considerably longer than for many other coastal bays (Kjerfve et al., 1997). Inputs of pollution may lead to long-term cumulative effects given the slow water exchange in the Bay. However, the sampling site is close to the entrance of the Bay, where the water turnover is faster than in other parts of the

Table 2. Maximum and minimum mean values for tissue *N*, *P* and *N:P* atomic ratio of ten seaweeds from Guanabara Bay, in 29 seasonal sampling. Data represent percentage of the dry weight, except for (*N:P*) atomic ratio (no units).

Species	Tissue *N*		Tissue *P*		(*N:P*) ratio	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
B. radicans	9.04 ± 0.83 Win 2001	6.65 ± 0.18 Win 2003	0.79 ± 0.01 Sum 2000	0.30 ± 0.04 Spr 2003	49.7 ± 5.48 Spr 2003	21.9 ± 0.53 Sum 2000
C. anteninna	5.26 ± 0.34 Aut 1998	3.04 ± 0.28 Win 2001	0.48 ± 0.05 Spr 2000	0.26 ± 0.01 Sum 2001	27.7 ± 0.67 Win 2002	14.5 ± 1.77 Spr 2001
C. decorticatum	5.27 ± 0.56 Win 1997	2.71 ± 0.23 Win 2002	0.65 ± 0.07 Spr 1997	0.22 ± 0.01 Spr 2002	42.4 ± 7.02 Spr 2001	17.2 ± 1.19 Win 2000
C. rupestris	6.92 ± 0.80 Win 2001	2.42 ± 0.17 Win 2003	0.66 ± 0.04 Win 1998	0.22 ± 0.01 Aut 2003	37.6 ± 5.46 Aut 2001	15.0 ± 1.62 Aut 2002
C. teedii	5.62 ± 0.36 Win 1998	3.25 ± 0.20 Sum 2004	0.82 ± 0.04 Spr 1999	0.42 ± 0.02 Win 2001	25.2 ± 1.31 Spr 2003	13.6 ± 0.77 Spr 1998
E. flexuosa	7.75 ± 0.61 Win 1998	3.16 ± 0.30 Spr 2001	0.83 ± 0.05 Aut 1999	0.20 ± 0.02 Aut 2001	49.1 ± 6.78 Aut 2001	17.1 ± 1.17 Spr 2003
G. griffthisiae	7.08 ± 0.07 Spr 1999	3.65 ± 0.17 Spr 2003	0.64 ± 0.05 Win 1998	0.30 ± 0.02 Sum 2004	38.1 ± 4.38 Sum 2004	17.9 ± 1.36 Aut 2001
G. doryphora	9.35 ± 0.17 Aut 1997	5.23 ± 0.26 Win 2003	0.74 ± 0.05 Spr 1997	0.26 ± 0.04 Sum 2004	49.8 ± 5.05 Sum 2004	18.0 ± 1.75 Spr 1997
U. fasciata	8.33 ± 0.38 Spr 2003	5.29 ± 0.76 Spr 2001	0.64 ± 0.05 Aut 1997	0.35 ± 0.02 Aut 2002	44.7 ± 3.59 Aut 2001	23.4 ± 1.80 Spr 1998
U. lactuca	7.51 ± 0.38 Spr 2002	4.10 ± 0.32 Win 2001	0.76 ± 0.02 Win 1999	0.28 ± 0.01 Spr 2001	56.8 ± 2.49 Spr 2001	19.7 ± 1.02 Win 1999

Data represent the mean of four determinations ± standard deviation ($n = 4$).

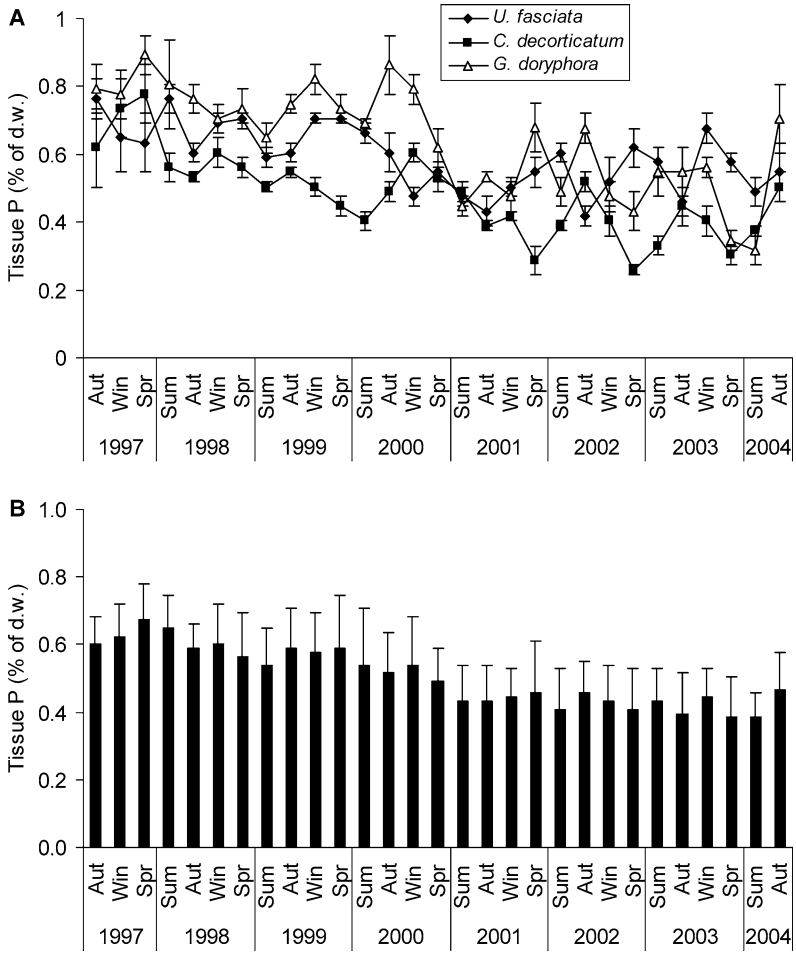

Figure 3. Seasonal fluctuations in the content of phosphorus in *Codium decorticatum*, *Grateloupia doryphora*, and *Ulva fasciata* (A), and mean values of *P* in the tissues of all macroalgae (B) collected in 29 seasonal samplings in Guanabara Bay. Data are expressed as percentage of the dry weight (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 112 to 144 measurements ± standard deviation ($112 \leq n \leq 1.44$).

system. Increased flushing facilitates the replacement of the nutrient-rich polluted waters with nutrient-poor oceanic waters every tidal cycle (Paranhos et al., 2001). This means that the impact of sewage-derived pollutant at the sampling site is substantially lower that in other parts of the Bay. This is probably one of the reasons for the greater species diversity of seaweeds at the study site than in the inner parts of the Bay (Teixeira et al., 1987).

Phytoplankton of Guanabara Bay is dominated by small-sized species (cyanobacteria and nanoplanktonic species), which are strong competitors for nutrients and achieve high biomass (Valentin et al., 1999). Shading by phytoplankton and particulate matter in deeper waters and the lack of rocky substrates in shallow areas restrict the proliferation of seaweeds (Mayr et al., 1989). These factors make the macroalgal biomass in the Bay relatively small compared to other eutrophic systems.

According to the Björnsäter & Wheeler's (1990) classification of macroalgal nutrient status 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 $N{:}P > 24$ indicates P-limitation. Applying this classification to our data we conclude that the macroalgal community in the sampling site is permanently N-sufficient and almost permanently P-deficient, with few exceptions. However, the $N{:}P$ ratio must be evaluated with care, as it may obscure trends for the individual elements. The

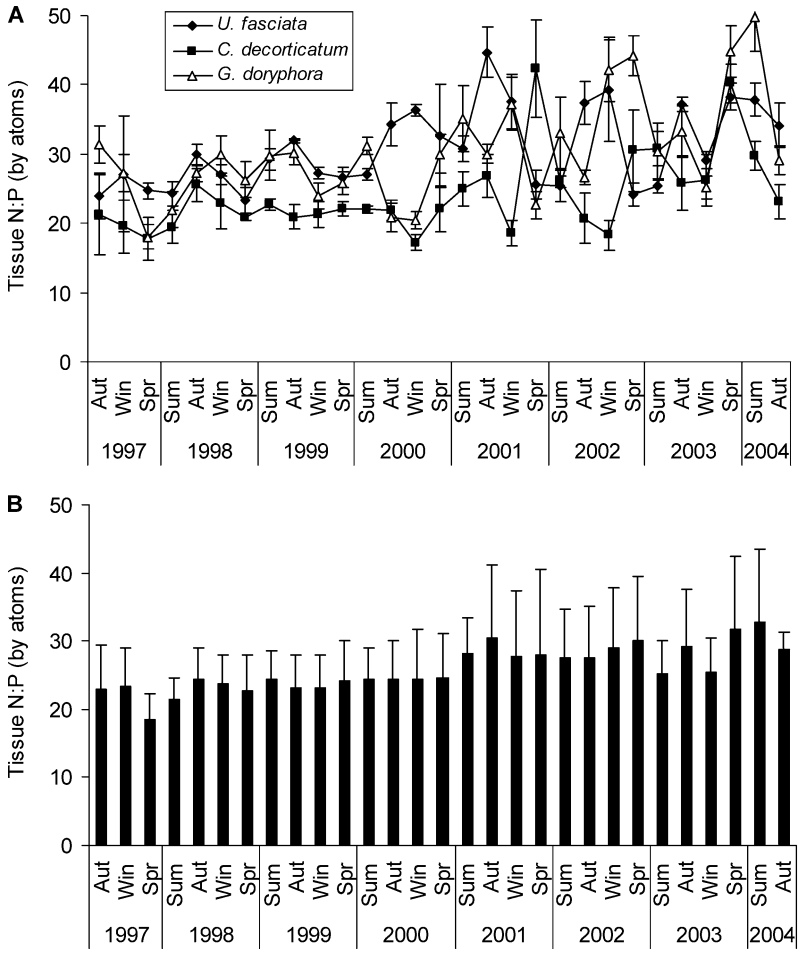

Figure 4. Seasonal fluctuations in tissue (*N:P*) ratio in *Codium decorticatum*, *Grateloupia doryphora*, and *Ulva fasciata* (A), and mean values of *N:P* in the tissues of all macroalgae (B) collected in 29 seasonal sampling in Guanabara Bay. In (A) each point represents the mean of four to six replicates ± standard deviation ($4 \leq n \leq 6$). In (B) each bar represents the mean of 112 to 144 measurements ± standard deviation.

overall mean values for tissue nitrogen and phosphorus in all algae collected in summer 2004 were 5.52 ± 1.69 and 0.38 ± 0.07% of d.w. ($n = 43$), respectively: the lowest mean value for phosphorus throughout this study. Howevere, 0.38% of tissue *P* does not represent a low level, and is actually higher than values found in many other algae from tropical environments (See Fong et al., 2003; Hwang et al., 2004). The high mean overall *N:P* ratio observed in Guanabara Bay in summer 2004 (32.7 ± 10.7, $n = 43$) is strongly affected by the high concentrations of nitrogen and is not necessarily indicative of *P* limitation. Thus, the classification of Björnsäter and Wheeler (1990) must be considered with caution, because the ranges may not be suitable for macroalgae from polluted tropical environments such as Guanabara Bay. In addition, further investigations are needed to test the suitability of that classification for tropical environments, where seaweeds typically grow well with low concentrations of dissolved nutrients and normally have lower tissue *N* and *P* compared to species from temperate environments. In this context the high *N:P* obtained for most of our measurements (typically >24:1) may not represent limitation of macroalgal growth by *P* at the study site.

A comparison of tissue nutrients and dissolved nutrients shows large differences in terms of *N:P* ratio, with higher values for the seaweeds. This apparent contradiction may be explained by the very limited usefulness of our data on dissolved nutrients. Monitoring dissolved nutrients is time-demanding and a reliable assessment needs a large data set, since many variables affect the results. In addition, our field samples were

always collected at low tides, when local sources of pollution at the site would be concentrated in the waters. Despite the construction of the Icaraí Submarine Sewage Outfall many sources of domestic sewage still exist and release their contents directly into the Bay. This still happens closer than 100 m to the sampling site. For this reason, our measurements of dissolved nutrients do not represent exactly the actual dynamics of nutrients at the sampling site.

The design of this descriptive study does not allow us to identify clearly the effects of the local sewage outfall since 1999. Our measurements of dissolved nutrients at the sampling site show no obvious pattern over the 7-year assessment (Table 1). However, other studies at different sites in the Bay have shown a significant decrease in the concentrations of dissolved nutrients following the construction of the Icaraí Submarine Sewage Outfall (unpublished data). If this is occurred at our site, it could explain the significant decrease of tissue *P* and increase of tissue *N:P* ratio of the species during this study.

In a related study, Lourenço et al. (2005) studied the seasonal variations of tissue *N* and *P* of eight macroalgal species of Araruama Lagoon, a hypersaline environment of Rio de Janeiro State. Remarkable seasonal variations in tissue nutrients for the seaweeds were found, with higher values in autumn and lower in spring for most of the species. The authors also considered that seaweeds are drastically affected by high temperatures in part of the spring and in the summer. Thermal damage could lead to the loss of tissue and nutrients to the environment (Hanisak, 1993; Menéndez et al., 2001). Typical summer temperatures in Guanabara Bay ($>25\,^{\circ}$C, data not shown) could potentially result in tissue loss. However, the available data do not support such an interpretation, because high values for tissue *N* and *P* were found during the warmest periods in spring and summer in some sampling. No seasonal variations for tissue nutrients were found, probably a consequence of permanent high concentrations of dissolved nutrients available to the species.

Levels of tissue *N* detected in the seaweeds suggest that the species are permanently saturated with nitrogen, even in periods when lower percentages of tissue *N* were measured. According to Hanisak (1979), maximum growth rate for the green alga *Codium fragile* can be achieved if the species has 2% of tissue nitrogen. Among the ten species studied here, the lowest tissue *N* value was 2.42%, measured in *Cladophora rupestris*, which suggests that the seaweeds did not experience limitation of growth by *N* at the site. The excess of nitrogen available for the seaweeds could stimulate most of them to a luxury consumption of nitrogen, generating high concentrations of tissue N, as demonstrated by Gordon *et al.* (1981) for *Cladophora* in cultures. Those authors determined that the critical tissue *N* and *P* concentrations for growth of *Cladophora* are 2.1% and 0.33%, respectively. Luxury consumption of nitrogen is more pronounced than the consumption of excess phosphorus (Gordon *et al.*, 1981), and this possible trend could account for the high *N*:*P* ratio seen in this study for most of the measurements done. This argument also points to the absence of phosphorus limitation in the site, especially because the values measured in the tissues were not low: tissue *P* was $>0.40\%$ for ca. 85% of all measurements. Our data indicate that seaweeds would not be limited by dissolved *N* and *P* in the site, and possible increments in algal biomass would be controlled by other factors such as herbivory (Lotze & Schramm, 2000) or lack of suitable substrate (Bokn et al., 2003).

In conclusion, we confirm that tissue *N* and *P* of the macroalgal species do not show any seasonal variation. In addition, tissue *N* and *P* of the species tested show high concentrations in most of the observations. However, our hypothesis regarding interannual consistency in tissue *P* and (*N:P*) ratio is rejected, as these have been decreasing over recent years.

We are currently evaluating the tissue *N* and *P* composition of many other seaweed species of Brazil, from coastal oligotrophic environments. These results will hopefully contribute towards a better understanding of the nutrient metabolism of tropical seaweeds.

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