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NITROGEN SOURCES TO MACROALGAL GROWTH IN SANYA BAY (HAINAN ISLAND, CHINA)

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Abstract

Nitrogen stable isotope ratios ($\delta^{15}N$) and molar C: N ratios were determined for 18 species of green, brown and red macroalgae and for 2 species of cyanobacteria collected at the Luhuitou coral reef (Sanya Bay, Hainan Island, China). Concentrations of dissolved inorganic nitrogen (DIN) and orthophosphates in seawater at the Luhuitou and the Xiaodonghai reefs were measured during different seasons. Composition of macroalgal and cyanobacterial communities was also investigated at these sites.

High $\delta^{15}N$ values of macroalgae (> 8‰) confirmed that wastes from the marine animal farms and sewages were the main sources of dissolved

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inorganic nitrogen (DIN) in Sanya Bay. The main source of nitrogen for cyanobacteria was determined as molecular nitrogen dissolved in seawater. Concentrations of orthophosphates and DIN in seawater above the reefs averaged 0.15 µM and 2.35 µM, respectively, exceeding the threshold concentrations (algal blooms) in 1.5-2.5-fold. It was shown that in the intertidal zone, mainly mono- and bidominant communities of crust algae and algal turf represent macroalgal communities. In the upper subtidal zone, both communities of algal turf and those of frondose and fleshy algae (*Sargassum* spp., *Padina* spp., *Turbinaria ornata*, *Lobophora variegata*, etc.) were common. The algae, especially, in the upper-subtidal zone, were tightly overgrown by epiphytes. The invasive species and algal blossom were not recorded.

We conclude that the main sources of DIN in the waters of Sanya Bay are human sewage or/and effluents from marine animal farms. In spite of high level of pollution by DIN in Sanya Bay, the molar C: N ratios in algal thalli were still at high level (on average >17) that give the ground to consider that algae of Luhuitou reef are limited in nitrogen. The latter may be explained by low photosynthetic rates in macroalgae over the difficulty in CO_2 and bicarbonate diffusion to the chloroplasts under abundance of sediments and epiphytes at the surface of algal thalli.

Introduction

Inputs of dissolved inorganic nitrogen (DIN, as ammonium, nitrate and nitrite) through sewage, fertilizer runoff or wastes of marine animal farms frequently increase the rate of primary production in coastal ecosystems [1, 2, 3], which can lead to large blooms of phytoplankton and/or macroalgae [4, 5, 6]. The macroalgal blooms can create large piles of decaying biomass on beaches when drift macroalgae is washed ashore and dies [7, 8]. Bloom forming species are typically characterized by thalli with large surface area to volume ratios (either thin and blade-like or filamentous and repeatedly branched), allowing for fast uptake of nutrients [9]. Blooms most commonly consist of green algae [10]. In Discovery Bay (Jamaica), groundwater inputs enriched the water column of the fringing reef by the increased nutrient concentrations in areas directly receiving groundwater discharges. High concentrations of DIN and soluble reactive phosphorus (SRP) promote high photosynthetic rate and biomass accumulation of not only the filamentous green alga *Chaetomorpha linum* but also frondose algae such as *Sargassum polyceratium*, *Lobophora variegata* and *Halimeda opuntia* [11]. In coral reefs, especially damaged earlier by bleaching or by other natural catastrophes, enrichment of waters with anthropogenic nitrogen promotes growth of seaweeds and over accumulation of their biomass in shallow areas that decrease competition ability of corals and prevent the recovery of the reef [12]. These effects can be amplified in areas of low water exchange, such as estuaries and partly enclosed bays [13].

Eutrophication of waters leads to the changes in composition of macroalgal associations. It is shown that ephemeral fast-growing green algae in the intertidal and upper subtidal zones displace slow-growing macroalgae. Frondose and fleshy algae quickly overgrow by epiphytes, which are able to take a leader state in biomass [14, 15, 16, 17].

Until recently, determination of DIN sources in coastal waters presented certain difficulty. At present, the sure test for DIN sources is a ratio between heavy ^{15}N and light ^{14}N stable isotopes in the tissue of plants assimilating DIN ($\delta^{15}N$ signature). The range of $\delta^{15}N$ values in marine environment extends from –3‰ to +46‰ [18]. DIN in sewage or waste of aquaculture farms has proportionally more ^{15}N than ^{14}N and thus a higher isotope ratio than DIN of oceanic waters ($\delta^{15}N$ ranges from +5‰ to +12‰) [19, 20, 21, 22, 23, 24]. At the same time, fertilizer runoff has DIN with the increased content of ^{14}N and the level of its $\delta^{15}N$ is lower than that in DIN of oceanic waters; it ranges from –31‰ to +3‰ [21, 22].

Investigations conducted last few years showed that macroalgae inhabiting clear oceanic waters have $\delta^{15}N$ signatures between +4.5‰ and +0.8‰ [25]; +2.95‰ and +2.51‰ [26]; +3.4‰ and +3.1‰ [27]; macroalgae inhabiting bays polluted by waters taken off from shrimp cultivation pounds have $\delta^{15}N$ signatures between +5.9‰ and +4.8‰ [28]; +6‰ and +4.2‰ [29, 30, 31]. Macroalgae inhabiting bays and estuaries with the inputs of sewage have the highest (>+6‰) level of $\delta^{15}N$ [26]; or between +16‰ and +7‰ [3]. The $\delta^{15}N$ values of the DIN absorbed and metabolized by the algae are not always equal or near to those recorded in the tissue of these algae, probably, due to discrimination of stable isotopes in the biochemical reactions of the algae. When nitrogen is present in excess in the seawater, the algae do preferentially take up the lighter isotope [20]. This isotopic discrimination may amount to several promilles (‰) for N in individuals and can confound the interpretation of isotopic ratios in tissue samples [32, 33, 34].

An analysis of $\delta^{15}N$ in algal tissue enables the determination of the main source of nitrogen (DIN) for macroalgae but not the concentration of DIN in the surrounding waters. The concentration of nitrous substances in water may be determined by direct methods only, and the more often water samples are taken, the more precisely dynamics of changes in nutrient (nitrous and phosphate compounds) concentration in water is defined during the day or the year.

The molar C: N ratio in the macroalgal tissue may inform both on the deficiency of nitrogen in the environment or on the lack of nitrogen limitation. The molar C: N ratio in most phytoplankton is 6.6 [35], when nutrients are not limiting. In tropical oceanic clear oligotrophic waters that are usually nitrogen limited, the C: N ratio in the tissue of macroalgal is reported to range from 14 to 28, and to vary by species [36].

We investigated composition and distribution of mass macroalgal species growing in Sanya Bay (Hainan Island, China), stable nitrogen isotope ratio and molar C: N proportion in the tissue of these algae and also nutrient enrichment of surrounding waters in the bay. We hypothetically proposed that water in Sanya Bay could be significantly polluted in DIN because of mariculture development and tourist business that should negatively influence on damaged (probably by last bleaching events) coral reefs and their restoration resulting in changes of algal communities structure with the increase of their competitive availabilities in comparison with corals. The aim of the present study was to elucidate the main source of water pollution by DIN in Sanya Bay, to determine the degree of water eutrophication and to estimate the influence of the eutrophication on nitrogen uptake by macroalgae and the structure of algal communities in the intertidal and subtidal zones of the Luhuitou coral reef.

Materials and Methods

Study site, time and conditions

Investigations were conducted at the Luhuitou reef, Sanya Bay, Hainan Island, China. The algae were collected in front of the Marine Biological Station of the South China Sea Institute of Oceanology in the intertidal and upper subtidal zones (up to 3 m depth) in April 2009. Water samples were collected at the adjacent Luhuitou and Xiaodonghai reefs from depths of 3 and 6 m in August 2008, in January and April 2009 (Figure). In the spring period, surface water temperature near the station was 24-28°C. Incident surface photosynthetically active radiation (PAR0) was 800-1600 μmol photons $m^{-2}s^{-1}$ at midday.

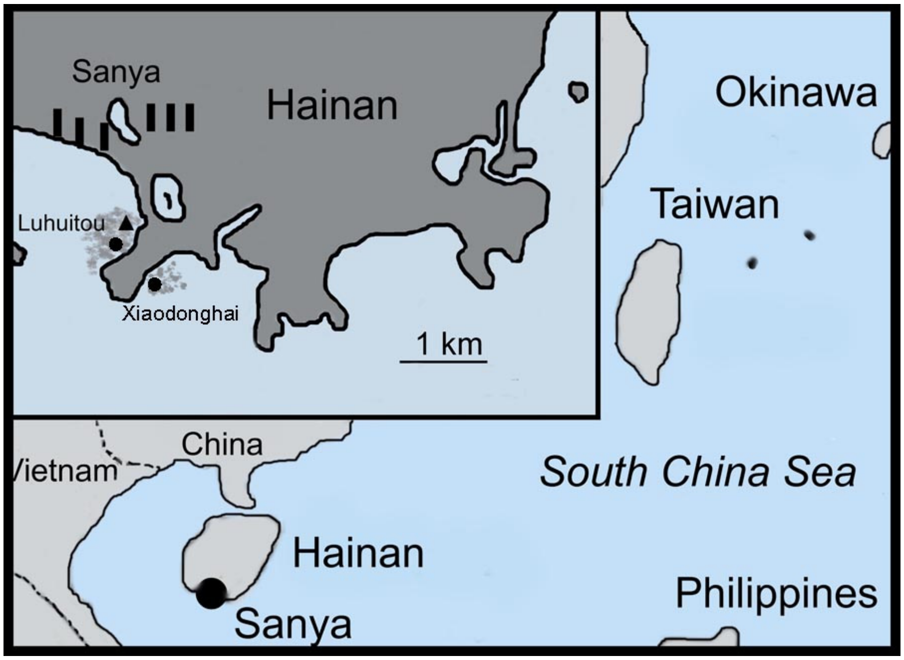

Figure. Study sites: ▲ – algal sampling sites; ● – water sampling sites.

Analysis of algal communities

Abundance of algal communities at the bottom was determined visually and by photographs using the relative value (%) of projective surface of the bottom occupied by the certain algal community or corals. Algal dominance in the community was determined visually. Algal community was defined as mono-dominant if one algal species occupied more than 50% of the bottom surface area where the community occurred. In bidominant community, two species occupied more than 50% of the bottom surface area. Other communities were defined as polydominant.

$\delta^{15}N$ analyses

For analysis of nitrogen stable isotope composition ($\delta^{15}N$), samples of macroalgae and cyanobacteria were washed with fresh water, cleaned of epiphytes, dried at $60^\circ C$ and packed in aluminum foil. Dried samples were homogenized using an agate mortar and pestle, and weighed (1.5 mg) into tin capsules prior to analysis.

The nitrogen isotopic compositions of the samples were determined using a mass spectrometer (MAT 253, Finnigan) coupled online via Finnigan ConFlo III interface with elemental analyzer (FlashEA 1112, ThermoQuest).

Results are expressed in the standard δ unit notation as $\delta X(‰) = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$, where X is ^{15}N and R is $^{15}N/^{14}N$. These values are reported relative to the atmospheric N_2. The molar C: N ratio was determined using the same equipment.

Determination of ammonium, nitrate, nitrite and phosphate concentrations in seawater

For analysis of nutrients, seawater samples were collected according to the methods and sampling tools of "Specification for Oceanography Survey" (GB 12763-91, China). Seawater was collected by scuba diving and transferred to lab in 2h in icebox. After filtration with 0.45 μm Millipore filter, Seawater was preserved in $-20^\circ C$ until nutrients analysis in several days. Concentrations of DIN components and orthophosphates in seawater were measured using standard methods [37]. All parameters were analyzed using spectrophotometer.

Statistical analysis

Data were analyzed with the statistical package Statistics v.6.0 (StatSoft Inc., Tulsa, Oklahoma, USA). Comparisons between means were conducted using the *T*-test for independent samples (module of The Basic Statistics and Table) and over time using the Tukey's Studentized range (HSD) test (module ANOVA).

Results

$\delta^{15}N$ signature and C: N ratio in common species of macroalgae and cyanobacteria

The $\delta^{15}N$ values for all macroalgal species investigated averaged 8.3‰. The

$\delta^{15}N$ values for the blue-green algae *Lyngbya majuscula* and *Lyngbya* sp. were 0.6‰ and 1.1‰, respectively (Table 1). The green algae *Ulva fasciata* and *Ulva* sp. had $\delta^{15}N$ signatures of 9.0‰ and 8.3‰, respectively. For seven species of brown algae, $\delta^{15}N$ averaged 7.6‰, and for nine red algae it was 8.6‰. Among macroalgae investigated, the heaviest nitrogen $(\delta^{15}N = 10.4‰)$ was found in the red alga *Gracilaria salicornia*, and the lightest nitrogen $(\delta^{15}N = 6.7‰)$ was observed in the brown alga *Lobophora variegata*.

The average value of the molar C: N ratio in macroalgal species was 19.8. In Cyanobacteria, it was twice lower, with means of 7.8 for *L. majuscula* and those of 8.1 for *Lyngbya* sp. On average, the most enriched in nitrogen species among macroalgae were the representatives of red algae (C: N = 16.4), especially, it was evident for *Acanthophora muscoides* (C: N = 11.5) and for *Acanthophora spicifera* (C: N = 11.8). The highest values of the C: N ratio were observed in the brown algae *Turbinaria ornata* (36.8), *Sargassum polycystum* (22.3) and *S. ilicifolium* (27.0) (Table 1). For the red algae, the highest C: N ratio was in *Palisada papillosa* $(\text{mean} = 23.4)$ and *Amphiroa fragilissima* $(\text{mean} = 21.6)$.

Table 1. $\delta^{15}N$ and C: N ratio in the tissue of macroalgae and cyanobacteria collected in Sanya Bay at the Luhuitou reef in April 2009

Species	$\delta^{15}N$	C: N	Collection site
Chlorophyta			
Ulva clathrata	9.0	17.5	intertidal zone
Ulva fasciata	8.3	21.1	intertidal zone
Average ± SD	8.7 ± 2.5	19.3 ± 2.5	
Heterokontophyta			
Sargassum ilicifolium	7.9	27.0	subtidal zone
Sargassum polycystum	8.2	22.3	subtidal zone
Turbinaria ornata	7.6	36.8	subtidal zone
Padina boryana	7.3	18.3	subtidal zone
Lobophora variegata	6.7	21.7	subtidal zone
Dictyota sp.	7.7	16.3	subtidal zone
Sphacelaria tribuloides	7.9	17.5	subtidal zone
Average ± SD	7.6 ± 0.5	22.8 ± 7.1	
Rhodophyta			
Liagora ceranoides	9.7	13.2	subtidal zone
Acanthophora spicifera	7.9	11.8	subtidal zone
A. muscoides	8.6	11.5	subtidal zone
Halymenia maculata	7.7	20.0	subtidal zone
Gracilaria salicornia	10.4	19.8	intertidal zone
G. tenuistipitata	9.5	14.5	intertidal zone
Palisada papillosa	7.7	23.4	subtidal zone
Average ± SD	8.7 ± 1.0	16.4 ± 4.7	
Species Cyanobacteria	$\delta^{15}N$	C: N	Collection site
Lyngbya majuscula	0.6	7.8	subtidal zone
Lyngbya sp.	1.1	8.1	subtidal zone

DIN and orthophosphate levels in Sanya Bay

DIN and orthophosphate concentrations in seawater from Sanya Bay are presented in Table 2. During the period from August 2008 to April 2009, the lowest DIN contents were observed in seawater samples collected at a 3 m depth at the

Luhuitou reef (mean = 1.28 μM) and at a 6 m depth at the Xiaodonghai reef (mean = 0.84 μM) in August. The highest DIN contents were recorded at a 3 m depth at the Luhuitou reef (mean = 3.93 μM) in January and at a 6 m depth at the Xiaodonghai reef (mean = 3.73 μM) in April. There were no constant significant differences in the DIN content between sites of 3 m and 6 m depths at either of the reefs investigated.

At the Luhuitou reef, the ammonium (NH_4^+) concentration in seawater did not depend on a season. The lowest (on average between depths was 0.15 μM) and the highest (on average between depth was 1.72 μM) nitrate concentrations were observed in seawater samples collected in August and in January, respectively. The highest (mean = 0.35 μM) nitrite concentration was recorded in January as well (Table 2).

Table 2. Concentration of dissolved inorganic nitrogen (DIN) and orthophosphates (μM) in seawater from Sanya Bay at different depths and during different seasons

Season	Depth	NO_4^+	NO_3^-	NO_2^-	DIN	PO_4^{-3}
		μM				
Luhuitou reef						
August 2008	3	1.10 ± 0.04	0.08 ± 0.02	0.06 ± 0.00	1.28	0.05
	6	2.00 ± 0.06	0.22 ± 0.03	0.12 ± 0.01	2.34	0.12
January 2009	3	1.70 ± 0.05	1.91 ± 0.03	0.31 ± 0.01	3.93	0.30
	6	1.43 ± 0.05	1.53 ± 0.03	0.39 ± 0.02	3.36	0.39
April 2009	3	1.87 ± 0.05	0.47 ± 0.03	0.04 ± 0.00	2.38	0.04
	6	1.21 ± 0.04	0.48 ± 0.04	0.11 ± 0.01	1.80	0.11
Average ± SD		1.54 ± 0.36	0.78 ± 0.75	0.17 ± 0.17	2.51 ± 0.97	0.17 ± 0.14
Xiaodonghai reef						
August 2008	3	0.09 ± 0.03	0.11 ± 0.02	0.06 ± 0.03	1.07	0.06
	6	0.65 ± 0.02	0.13 ± 0.02	0.06 ± 0.03	0.84	0.06
January 2009	3	1.23 ± 0.04	0.94 ± 0.01	0.20 ± 0.01	2.38	0.20
	6	0.90 ± 0.03	0.86 ± 0.01	0.22 ± 0.02	1.99	0.23
April 2009	3	2.52 ± 0.07	1.00 ± 0.09	0.15 ± 0.02	3.67	0.14
	6	2.73 ± 0.07	0.92 ± 0.08	0.07 ± 0.02	3.73	0.07
Average ± SD		1.48 ± 1.05	0.66 ± 0.42	0.13 ± 0.07	2.28 ± 1.24	0.13 ± 0.07

At the Xiaodonghai reef, the highest concentrations of NH_4^+, NO_3^- and NO_2^- in seawater were observed in April and January (Table 2).

Orthophosphate (PO_4^{-3}) levels were about 0.17 µM at the Luhuitou reef and about 0.13 µM at the Xiaodonghai reef. At both reefs investigated, the highest PO_4^{-3} concentrations were recorded in January 2009 (Table 2).

Algal communities in the intertidal and upper subtidal zones of the coral reef

The hard substrate (stones, dead coral colonies, carbonate basis of the reef) in the upper, middle and low intertidal zones was occupied mainly by macroalgae and cyanobacteria. Living scleractinian corals were not found in the upper and middle intertidal zones. In the upper subtidal zone macroalgae and cyanobacteria occupied substrate to 50-90%, scleractinian corals covered the bottom up to 5-40%. Algae were not found in the splash zone.

In the intertidal zone, macroalgae formed two types of communities: monodominant community of crust algae and monodominant, bidominant or polydominant communities of turf-forming algae. In the upper subtidal zone, polydominant communities of macroalgae prevailed. Substrate in the upper intertidal zone was represented mainly by gravel and boulders consisted of stones and coral debris. Large coral boulders were occupied by monodominant community of the crust alga *Ralfsia* sp. and by monodominant communities of algal turf with the dominance of the red alga *Lomentaria corallicola* and the green alga *Ulva clathrata*. Accompanying species in the algal turf community of *L. corallicola* were represented by the green alga *Cladophora laetevirens*, by the red algae *Parviphycus adnatus* and *Gelidium pusillum* var. *cylindricum*, and by the blue-green algae *Lyngbya semiplena* and *L. majuscula*. The surf zone was represented by the belt (50 cm in width, 5 cm thick) of junk algae among which *Ulva fasciata* was a dominant species and *Acanthophora spicifera*, *Hypnea pannosa*, *Halymenia maculata*, *Gelidiella acerosa*, *Padina boryana*, etc. were common.

In the middle intertidal zone, algal turf community was presented by dominant *Polysiphonia japonica* var. *savatieri*, which occupied mainly flat carbonate reef basis. The red algae *Herposiphonia secunda f. tenella*, *Hypnea spinella*, *Spyridia filamentosa*, *Centroceras clavulatum* and *Jania capillacea* were common among the bed of *P. japonica* var. *savatieri*. Monodominant community of *Ulva clathrata* occupied large boulders, where main accompanying species were *P. adnatus*, *H.*

secunda f. *tenella*, *P. japonica* var. *savatieri*, *Ceramium camouii*. At the border of the low intertidal zone, mainly bidominant community of *Ulva clathrata* and *C. laetevirens* occupied the reef basis, where *Ulva fasciata* and *Palisada papillosa* were common species. The community of turf algae occupied the reef basis and large coral debris where dominant species were *Parviphycus tenuissimus* and *P. papillosa*. In this community, *Ulva fasciata* and *Sphacelaria novae-hollandiae* were frequently found.

In the middle intertidal zone, monodominant community of the crust red alga *Hildenbrandia rubra* was common on the upper surface of stones. On the shaded bottom surface of the coral boulders, monodominant community of the red crust algae *Lithothamnion* sp. was common.

Mostly turf algal communities occupied the low intertidal zone, where *Acanthophora spicifera*, *P. papillosa*, *Tolypiocladia glomerulata* were dominate. Furthermore, the algae such as *Acanthophora muscoides*, *Hypnea valentiae*, *Hydropuntia eucheumatoides*, *Spyridia filamentosa*, *Galiyella flaccida* [*Ceramium flaccidum*], *Colpomenia sinuosa*, *Hydroclathrus clathratus*, *Padina boryana*, *Boodlea composita*, *Caulerpa serrulata* were also common. Communities of Cyanobacteria often covered the algal turf where *Oscillatoria agardhii* and *Lyngbya majuscula* were dominant.

In the upper subtidal zone, two algal communities were found: bidominant community of the brown frondose algae *Sargassum polycystum* and *S. ilicifolium* represented one of them. At the edge of the *Sargassum* bed, *S. polycystum* was densely overgrown by epiphytes, such as *Acrochaetium robustum*, *Centroceras clavulatum*, *G. flaccida*, *Ceramium comptum*, *C. sympodiale*, *Polysiphonia japonica* var. *savatieri*, *Hincksia mitchelliae*, *Sphacelaria tribuloides*. The community was distributed along foreshore at the border with the low intertidal and upper subtidal zones.

Behind the bed of *Sargassum* species, the hard substrate consisted of the patch reef debris and was occupied by polydominant community of turf algae where *Hypnea pannosa* and *H. valentiae* were the main turf-forming algae. In the turf, *A. spicifera*, *Amphiroa foliacea*, *Ceratodictyon spongiosum*, *Gelidiella acerosa*, *Halymenia maculata*, *Jania capillacea*, *Peyssonnelia conchicola*, *T. glomerulata*, *Dictyota friabilis*, *B. composita*, *Bornetella oligospora*, *B. sphaerica*, *Bryopsis pennata*, *Caulerpa serrulata*, *Neomeris annulata* were common. Scleractinian corals from the genera *Porites*, *Acropora*, *Pocillopora*, *Stylophora*, *Montipora*, etc. were also common in the upper subtidal zone.

Discussion

$\delta^{15}N$ signature and C: N ratio in common species of macroalgae and cyanobacteria

The $\delta^{15}N$ signatures of thalli of all macroalgal species investigated in Sanya Bay varied between 6.7‰ and 10.4‰. The average $\delta^{15}N$ values did not differ between green, red and brown algal groups, suggesting either sewage water of Sanya city or/and effluent from marine animal farms as the main DIN sources for the algae. Recently conducted studies show that macroalgae inhabiting bays and estuaries with the water inputs of human sewage, have a $\delta^{15}N$ signature of > 6‰ [26] or > 9‰ (Titlyanov, unpublished data), or in a range from 7‰ to 16‰ [3, 55]. The algae growing in bays polluted by water inputs from shrimp farms have $\delta^{15}N$ signature of 4.8 to 5.9‰ [28] and 4.2 to 6.0‰ [29, 30, 31]. In contrast, the algae inhabiting clear areas of the sea have feature $\delta^{15}N$ signatures of 0.8 to 4.5‰ [25]; 2.51 to 2.95‰ (26); 3.1 to 3.4‰ [27] and 2.2 to 2.5‰ (Titlyanov, unpublished data).

Cyanobacteria *Lyngbya majuscula* and *Lyngbya* sp. show $\delta^{15}N$ values of about 1.0‰. These $\delta^{15}N$ signatures suggest that these species are actively fix molecular nitrogen dissolved in seawater.

In the tissue of macroalgae investigated, the molar C: N ratio varied between 11.5 (*Acanthophora muscoides*) and 36.8 (*Turbinaria ornata*), and it was on average 19.3, 23.7 and 16.4 for green, brown and red algal groups, respectively. In Cyanobacteria, the C: N ratio was about 8.0 (Table 1). As was shown earlier, the molar C: N ratio in most phytoplankton is 6.6, when nutrients are not limiting, and it is essentially increased under nitrogen limitation [35]. In tropical oceanic clear oligotrophic waters, nitrogen is limited for non-nitrogen-fixing autotrophic organisms. In the tissue of macroalgae grown in the nitrogen depleted waters, the C: N ratio is reported to increase and ranges from 14 to 28 [36].

Lapointe et al. [36] determined the threshold value of the C: N ratio as 14 that point out at the seawater deficiency in DIN for maintenance of normal algal growth. If this threshold value of the C: N ratio will be shift down to 12, than we may find that all the algae investigated are nitrogen limited under the conditions of the Luhuitou reef, and only blue-green algae are able to acquire and assimilate sufficient amounts of nitrogen.

DIN and phosphate contents in waters of Sanya Bay

In seawater of Luhuitou and Xiaodonghai reefs in Sanya Bay, nutrient levels from this study (Table 2) varied, mainly, in respect to seasons (DIN was in a range of $0.84 - 3.93\,\mu\text{M}$; ammonium $-0.65 - 2.73\,\mu\text{M}$; nitrate $-0.08 - 1.93\,\mu\text{M}$; nitrite $-0.04 - 0.39\,\mu\text{M}$). The lowest DIN content was recorded in August 2008, and the highest DIN was observed in January and April 2009. The content of phosphates varied in a range from 0.04 to 0.39 μM, with the highest values observed in January 2009. These findings suggest that most of the year, seawater was sufficiently enriched with dissolved nitrogen and phosphorus accessible for plants at the sites investigated in Sanya Bay. For comparison, in oceanic atolls of the Great Barrier Reef, Australia, seawater contained 0.05 μM of total oxidized nitrogen TON $(NO_3^- + NO_2^-)$ and 0.08 μM of soluble reactive phosphorus, SRP [40]. In Tikehau Atoll (French Polynesia), seawater TON and SPR contents were in a range of $0.03 - 0.06\,\mu\text{M}$ and $0.10 - 0.11\,\mu\text{M}$, respectively [41, 42]. The DIN levels in the seawater of Luhuitou and Xiaodonghai coral reefs were similar to those recorded for the Red Sea (Egypt) at near shore reefs of Safaga and Quseir $(0.73 - 1.86\,\mu\text{M})$ with sewage inputs [10]; at reefs of the Florida Keys (USA) $(1.07 \pm 0.56\,\mu\text{M})$ with Florida Bay inputs [43]; at coral reefs in Southeast Florida $(0.43 - 0.89\ \mu\text{M})$ with groundwater inputs [11]; at a high-latitude coral reefs of Western Australia in the Houtman Abrolhos Islands $(0.83 - 1.50\ \mu\text{M})$ with sediments mineralization [44]; at the southwest coast of Martinique, Caribbean $(0.53 - 0.62\ \mu\text{M})$ near-urban area [45]. The seawater DIN concentrations higher than in Sanya Bay were recorded at reefs of Discovery Bay in Jamaica $(4.61 - 20.65\ \mu\text{M})$ with groundwater inputs [11]; at the fringing reefs of Guam $(8.04 - 5.75\ \mu\text{M})$ with terrestrial runoff [46]; at the Guarajuba reef in Brazil $(6.09 - 8.19\ \mu\text{M})$ near urban areas [47].

Concentrations of phosphate in the seawater of Luhuitou and Xiaodonghai reefs were approximately twice higher than those in the seawater of oceanic atolls, $\text{RSP} = 0.10 - 0.11\ \mu\text{M}$ [41, 42] and at the Great Barrier Reef, $\text{RSP} = 0.08\ \mu\text{M}$ [40]; but were close to the phosphate values registered at the reefs of Discovery Bay in Jamaica, RSP = 0.26 – 0.13 μM, with groundwater inputs [11] and at inshore reefs of the Florida Keys (USA), RSP = 0.11 – 0.17 μM [43].

Algal communities

According to the floristic analysis conducted in the intertidal and upper subtidal zones of the Luhuitou reef, marine macroalgae occupied mostly the hard substrate: rocky bottom, carbonate reef basis, dead coral colonies and their debris. Living scleractinian corals were observed as patch reefs or in between algal turf only in the upper subtidal zone. Mainly algal turf and crust algae represented intertidal algal communities. In the subtidal zone, fleshy, frondose and foliose algae enriched communities of algal turf. *Sargassum* spp. community occupied the upper border of the subtidal zone.

Finely branched, hard articulate filamentous red and green algae represented turf-forming species. In the upper intertidal zone, these species were represented by *Lomentaria corallicola* and *Ulva clathrata*; in the middle intertidal zone – *Polysiphonia japonica* var. *savatieri*, *Palisada papillosa*, *Parviphycus tenuissimus*, *U. clathrata* and *Cladophora laetevirens*; in the low intertidal zone – *Acanthophora spicifera*, *P. papillosa* and *Tolypiocladia glomerulata*. In the upper subtidal zone, community of frondose and fleshy algae were presented by dominant algae such as *Sargassum polycystum*, *S. ilicifolium*, *Hypnea pannosa*, *H. valentiae*, *Lobophora variegata* and *Padina boryana*. Moreover, skeletons of dead corals were overgrown by algal turf consisting of dominant species such as *T. glomerulata*, *Centroceras clavulatum*, *Galiyella flaccida*, *Sphacelaria novae-hollandiae*, *Herposiphonia secunda* f. *tenella*.

The number species of red algae was highest in the locations investigated. At the same time, brown algae such as *Sargassum* spp. had the highest total biomass. The casts of the algae ashore were minor. The casts of the algae consisted of low intertidal and upper subtidal attached algae inhabiting the reef investigated. Invasive species were not found. Thus, Luhuitou reef by the nature of its algal communities (turf, crusts, frondose, fleshy algal communities, temporary communities of Cyanobacteria), composition of macroalgae and cyanobacteria and the projective surface area of the bottom occupied by algal communities, is similar to that of damaged coral reefs of Australia [38], Okinawa [39] and Con Dao Islands in Vietnam (Titlyanov and Titlyanova, unpublished data).

Conclusions

We conclude that DIN and phosphate levels in the seawater of Luhuitou and Xiaodonghai reefs (as most likely across all Sanya Bay) are higher (3-5-fold and 10-fold, respectively) than those in clear waters of insular coral reefs. The main sources of DIN in the waters of Sanya Bay are human sewage or/and effluents from marine animal farms. Such a high concentration of nutrients in Sanya Bay may induce both phytoplankton and macroalgal blooms. It is evident that reefs exposed to chronic nutrient enrichment increase their primary productivity, mainly due to expansion of macroalgae [48, 49, 50, 51, 11]. Furthermore, coral reefs are particularly susceptible to nutrient enrichment in consequence of very low thresholds for DIN (1.0 μM) and SRP (0.1 μM) [49]. These DIN and SPR levels were noted as the threshold concentrations for degradation of coral reefs from eutrophication and subsequent macroalgal blooms at Kaneohe Bay in Hawaii, fringing reefs of Barbados and inshore reefs within the lagoons of the Great Barrier Reef [52]. These concentrations also represent nutrient thresholds experimentally determined by Lapointe et al. [52] for macroalgal overgrowth of seagrasses and coral reef communities. In coral reefs, concentrations of nutrient substances above the threshold were reported to induce growth and accumulation of biomass by mainly frondose macroalgae [52], provoking superabundant macroalgal blooms. However, floristic survey at the Luhuitou reef showed that algal communities and algal species that are typical for tropical coral reefs occupied this site. We did not observe superabundant large frondose macroalgae, such as *Sargassum* spp., *Lobophora variegata*, *Dictyota spp.*, *Halimeda* spp. that were reported by Lapointe et al. [52] as the species widely distributed at the nutrient enriched reefs in Jamaica. However, at the Luhuitou reef, we observed epiphyte blooms, especially, common for macroalgae such as *Sargassum* spp., *Padina* spp., and *Dictyota* spp. We suggest that species composition and biomass of macroalgae and their epiphytes, as well as those of cyanobacteria and corals at the Luhuitou reef are controlled by the action of predators and resources availability (“top-down” and “bottom-up”) [53, 54, 9]. We conclude that at the time of this study, Luhuitou reef was in a steady-state condition and adapted to the increased concentrations of nitrogen and phosphorus substances.

Analyses of the molar C: N ratio in the tissue of macroalgae inhabiting the Luhuitou reef showed that they were nitrogen limited besides the high seawater DIN concentration, suggesting a limited ability of the algae to absorb dissolved inorganic nitrogen. This is theoretically in account with either some difficulty of nitrogen access to the attractive centers or with the seawater deficiency in elements or substances contributing to nitrogen absorbance, or with the presence of nitrogen assimilation inhibitors in the polluted waters of Sanya Bay. We suggest that assimilation of seawater nutrients by macroalgae is prevented by low photosynthetic rates of the algae over the limitation in CO2 and bicarbonate diffusion to chloroplasts under the thick layer of sediments covering the algal thalli and the dense overgrowth of thalli by epiphytes. However this hypothesis needs an accurate experimental verification both in the field and laboratory experiments.

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