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1 **Planktonic and Benthic Microalgal Community Composition as**  
2 **Indicators of Terrestrial Influence on a Fringing Reef in Ishigaki**  
3 **Island, Southwest Japan**

4  
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12

13 **Abstract** Microalgal-based indicators were used to assess terrestrial influence on Shiraho  
14 coral reef of Ishigaki Island (Okinawa, Japan). A typhoon occurred on 4-5 August 2005 and  
15 sampling were made on three occasions thereafter (6, 8, 11 August). Pre-typhoon sampling  
16 was conducted on 26 July. The typhoon-enhanced terrestrial discharges increased reef nutrient  
17 levels (e.g. average NO<sub>3</sub><sup>-</sup>-N: 0.088 mg/L pre-typhoon to 0.817 mg/L post-typhoon). This  
18 elevated chlorophyll-*a* concentrations by four times and shifted phytoplankton composition  
19 (spectral class-based) from an initial dominance of diatoms and green microalgae to the  
20 dominance of bluegreen microalgae (cyanobacteria) and cryptophytes. Cyanobacteria later  
21 increased by more than 200% and accounted for as much as 80% of total chl-*a* (~0.29 µg/L),  
22 possibly assisted by favorable nutrient availability. In outer reef waters, diatoms and green  
23 microalgae predominated whereas cyanobacteria and cryptophytes were nearly undetectable.  
24 Due to detrital decomposition and river discharge, the CDOM was much higher in the inner  
25 reef than in the outer reef. Benthic bluegreen microalgae were relatively more abundant in  
26 areas close to the river mouth and coastal agricultural fields. At these locations, nutrient

1 concentrations were much higher due to river discharge and potentially significant  
2 groundwater discharge. Thus, phytoplankton and benthic microalgae can serve as indicators  
3 of terrestrial influence on coral reefs.

4

5 **Keywords:** *Phytoplankton; Benthic microalgae; Bioindicators; Nutrients; Coral reef; Coastal*  
6 *waters; Terrestrial influence; Monitoring; CDOM*

7

## 8 **1. Introduction**

9 Assessing terrestrial influence on coral reefs is increasingly important as the degradation of  
10 coral reefs continues. Terrestrial runoff delivering excessive nutrients and sediments can have  
11 a variety of detrimental effects on the ecology of coral reefs. These include decreased coral  
12 cover, changes in coral community, increased algal growth (Fabricius, 2004) and proliferation  
13 of macroalgae and benthic cyanobacteria, which can adversely affect larval recruitment of  
14 corals (Kuffner and Paul, 2004). However, most watershed-coral reef studies have focused on  
15 physical (e.g., hydrology/hydrodynamics, sediment discharge) and chemical (e.g., nutrients)  
16 aspects; little attention has been devoted to monitoring algae (i.e., abundance, composition),  
17 despite its potential as an indicator of coastal eutrophication.

18 Eutrophication can lead to ‘phase shift’ or the transformation of a reef from being coral-  
19 dominated to algae-dominated. Early detection through monitoring can help prevent the  
20 occurrence of nuisance filamentous algae and other deleterious effects on corals. Integrated  
21 methodologies to assess coastal ecosystem condition and eutrophication status are warranted.

22 A new set of ecologic indicators must be developed to fully measure coastal system  
23 complexities (Niemi et al., 2004). Proposed indicators include biological community  
24 approaches to detect effects on ecosystem structure and function. Microalgae are known to  
25 respond to changes in environmental conditions (e.g., nutrient availability, sedimentation,  
26 hydrology, irradiance, and temperature regimes) over a wide range of temporal scales (e.g.,

1 hours to even years). In addition, ‘the ecological effects of environmental stressors are often  
2 evident at the microbial level, where the bulk of primary production and biogeochemical  
3 recycling occurs’ (Paerl et al., 2003). However, information on the influence of environmental  
4 perturbations on changes at the base of the food web that mediate productivity and nutrient  
5 cycling are scarce (Paerl et al., 2003). Chlorophyll-*a* (*chl-a*) is the most commonly used  
6 indicator of eutrophication. However, since *chl-a* is a bulk indicator, it can only provide  
7 limited information and must be used with caution (Andersen, 2006). Various studies have  
8 suggested the use of microalgal functional groups or phytoplankton community composition  
9 as indicators of coastal ecosystem condition, since each group responds differently to nutrient  
10 availability and environmental conditions (Pickney et al., 2001; Paerl et al., 2003). Nutrient  
11 enrichment can result in changes in the phytoplankton community (Piehler et al., 2004),  
12 especially in areas subject to episodic nitrogen enrichment due to pulses of nitrogen-rich river  
13 discharge (Ornolfsdottir et al., 2004). However, little information is available on the  
14 phytoplankton community of coral reef waters (van Duyl et al., 2002; Tada et al., 2003).  
15 Differentiation of algal groups is also necessary to establish whether the reef environment  
16 influences phytoplankton composition (van Duyl et al., 2002).

17 Nutrient enrichment can also result in shifts in the benthic microalgal community (Armitage  
18 and Fong, 2004). Benthic microalgae, or microphytobenthos, consist of microscopic,  
19 photosynthetic eukaryotic algae and cyanobacteria (bluegreen microalgae) that inhabit the  
20 upper several millimeters of illuminated sediments (MacIntyre et al., 1996). Communities of  
21 benthic microalgae are ubiquitous and abundant in coastal marine sediments and are  
22 responsive to both light and nutrients (Barraquet et al., 1998; Heil et al., 2004). Growth of  
23 benthic microalgae can be enhanced by elevated levels of nitrogen (Dizon and Yap, 1999;  
24 Hillebrand et al., 2000) or phosphorus (e.g., Fong et al., 1993; Kuffner and Paul, 2001).  
25 Benthic microalgae, therefore, provide a potential means of assessing terrestrial influence  
26 through groundwater discharge.

1 Ishigaki Island is part of the Ryukyu Islands located in the southwest portion of Japan (Fig. 1).  
2 Shiraho coral reef, located on the island's east coast, has been subjected to various stresses  
3 like sedimentation (Omija et al., 1998; Mitsumoto et al., 2000) and high sea temperature  
4 (Fujioka, 1999), resulting in degradation of the coral reef. Terrestrial influence on Shiraho  
5 Reef is exerted mainly through the Todoroki River Groundwater discharge to the coral reef  
6 can be an important source of nutrients, considering the adjacent lands are usually overlaid  
7 with permeable bedrock like limestone (Umezawa et al., 2002a).

8 Fig. 1

9 Terrestrial influence on Shiraho Reef has been documented through the direct monitoring of  
10 nutrient distribution (Kawata et al. 2000, Umezawa et al. 2002) and macroalgal isotopic  
11 signatures (Umezawa et al. 2002). However, the microalgal community composition has not  
12 been investigated. In this study, the utility of microalgae as potential indicators of terrestrial  
13 influence on coral reef was examined. The spatial and temporal variation of planktonic  
14 microalgae in Shiraho Reef was evaluated using a submersible fluorometer. The relationship  
15 between microalgal concentration/composition and nutrient concentration/ratio in the reef was  
16 analyzed using exploratory statistical analysis. Benthic microalgae (concentration and  
17 composition) were measured on the nearshore reef areas during low tide to evaluate their  
18 spatial variation as affected by nutrients attributable to groundwater discharge into the reef.

19

20 **2. Materials and Methods**

21 **2.1 Study site**

22 Shiraho Reef is a well-developed fringing reef with typical topographic features such as moat,  
23 reef pavement, reef crest, and reef edge (Kayanne et al., 1995). Average water depth in the  
24 moat is about 2 m. The reef crest emerges at low tides, during which the average water depth  
25 in the moat is about 1.5 m (Nadaoka et al., 2001). The width of the fringing reef varies from  
26 0.7 to 1.4 km. Four channels exist in the reef, namely, 'Tooru-guchi', 'Ika-guchi',

1 ‘Moriyama-guchi’, and ‘Bu-guchi’ (Fig. 1). Tooru-guchi is the largest channel, penetrating  
2 deeply into the reef and having an average depth of 20 meters (Tamura et al., 2007). Two  
3 rivers drain into the Shiraho Reef, the Todoroki and Tooru Rivers. The Todoroki River  
4 supplies large amounts of sediment and nutrients from the Todoroki watershed. The Tooru  
5 River drains towards the Tooru-guchi channel. Seagrass and macroalgae proliferate in  
6 nearshore areas north of the river mouth. Corals are scattered throughout the reef flat and are  
7 abundant in the reef slope (outer reef). The typical flow circulation pattern (tide-averaged  
8 velocity) in the reef (Fig. 22a in Tamura et al., 2007) indicates an evident convergence of  
9 mean currents toward Tooru-guchi and subsequent flow offshore. Flows in and near  
10 Moriyama-guchi and Ika-guchi are mostly directed offshore. Most importantly, the flow  
11 pattern near the Todoroki River mouth indicates that the current is directed northward. Water  
12 flows towards Moriyama-guchi and further northwards to Tooru-guchi. The pattern of  
13 currents in Shiraho Reef under small wave conditions is almost the same as that during high  
14 wave conditions (Tamura et al., 2007), thus, the flow pattern described can be considered  
15 representative. Particle tracking simulations showed that suspended particles from Todoroki  
16 River mouth are transported towards Tooru-guchi (Tamura et al., 2007). These characteristics  
17 can also have significant influence on nutrient transport within the reef.

18  
19 The Todoroki watershed is a small (about 10.82 km<sup>2</sup>) watershed devoted to intensive  
20 agriculture, including livestock farming. Most of the land is planted to sugarcane and rice.  
21 The rainy season in Ishigaki starts in early or mid-May and lasts for about one month,  
22 followed by typhoon occurrences in July to October. Soil erosion and potentially excessive  
23 discharges of sediments and nutrients onto Shiraho Reef occur during these periods. Based on  
24 numerical simulation, 47% (24.13 tons) of detached sediments (51.34 tons) were discharged  
25 through the river outlet in four rainfall events in June 2000 (Paringit and Nadaoka, 2003). The  
26 Todoroki River discharges about 2,240 tons of suspended solids, 71.5 tons of nitrogen, and

1 6.6 tons of phosphorus annually (Nakasone et al., 2001). Numerous cow barns are located  
2 within the watershed and adjacent lands. The subsurface geology of the watershed is mainly  
3 gravel conglomerate, limestone, and alluvial formations.

4

## 5 **2.2 Methodology**

6 The overall methodology utilized in this study was an integration of various measurement and  
7 monitoring approaches employing different data-logging type instruments (e.g., optical,  
8 fluorometric), standard water sampling procedures, and subsequent analysis for nutrients.  
9 Field surveys were conducted at Shiraho reef and in the Todoroki watershed during summers  
10 of 2005 and 2006 in order to quantify nutrient loadings and responses of phytoplankton and  
11 benthic microalgae. However, only measurements made at Shiraho reef are presented here.

12

### 13 *2.2.1 Reef water quality monitoring*

14 Data-logging sensors were deployed at stations R1 to R12 of Shiraho Reef (Fig. 1) to monitor  
15 various hydrodynamic (e.g., velocity, wave height) and water quality parameters (e.g.,  
16 turbidity, chlorophyll-*a*) over a one-month period (July 23 – August 22, 2005). Details on  
17 instrument settings and deployment setups are available in Yamamoto et al. (2006). Stations  
18 R1, R4, R6, and R12 were located near the channels while the other stations were relatively  
19 closer to the shoreline. Turbidity and chlorophyll-*a* concentration were monitored at most  
20 stations using Compact-CLW (Alec Co., Japan) deployed just below the water surface with a  
21 moored buoy setup. Salinometers (Compact-CT, Alec Co., Japan) were also deployed using  
22 the same setup.

23

24 Periodic water sampling and profile measurements (using an STD-type instrument) were  
25 made at most stations (except R2 and R11) during low tides. Water samples were taken just  
26 below the water surface and from near bottom using a modified Niskin sampling bottle

1 (Yoshino Keisoku Co.,Ltd., Japan). Samples were stored in the dark in cooled containers  
2 while in the field. Samples for dissolved nutrient analysis were filtered through 0.45  $\mu\text{m}$   
3 acetate membrane filters (Advantec Dismic 25CS045AN). Filtrates were immediately frozen.  
4 Concentrations of dissolved nutrients ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , and  $\text{SiO}_2$ ) were later  
5 determined using a Bran-Luebbe TRAACS 2000 auto-analyzer (Bran and Luebbe,  
6 Norderstedt, Germany). For chlorophyll-a analysis, samples were filtered using Whatman  
7 GF/F (0.7- $\mu\text{m}$  pore size, 25mm diameter) glass microfiber filters. Chlorophyllous pigment  
8 was extracted using N,N dimethylformamide following the method of Suzuki and Ishimaru  
9 (1990). Chlorophyll-a content in the extract was then analyzed based on the method of  
10 Strickland and Parsons (1972) using a calibrated Turner fluorometer.

11

### 12 2.2.2 Measurement of planktonic microalgae

13 Class-differentiated algal measurements were conducted using a bbe FluoroProbe system (bbe  
14 Moldaenke, Kiel, Germany). This submersible instrument makes use of the fluorometric  
15 characteristics of microalgae to determine their class and concentration. FluoroProbe can  
16 differentiate phytoplankton into four spectral classes: 'green' algae (Chlorophyta), 'blue'  
17 algae (bluegreen algae or phycocyanin-containing cyanobacteria), 'brown' algae  
18 (Chromophyta, Dinophyta), and 'mixed' (Cryptophyta). The relative amount of colored  
19 dissolved organic matter (CDOM), also known as yellow substance, is measured to correct for  
20 the influence of the substance on microalgal fluorescence. Briefly, the instrument performs  
21 sequential light excitations using five light emitting diodes (LEDs) emitting at different  
22 wavelengths (i.e., 450, 525, 570, 590, and 610 nm) (Beutler et al., 2002). Subsequent  
23 measurements of relative fluorescence intensity of chl-*a* at 680 nm are then made  
24 immediately after each light excitation. By comparing the measured fluorescence excitation  
25 spectra or 'fingerprints' for the different algal classes with the calibration excitation spectra  
26 stored in the instrument, the relative amounts of each algal class are calculated using an

1 algorithm. The manufacturer-provided calibration data were obtained by quantifying chl-*a*  
2 amounts in mixtures of laboratory cultures using high-performance liquid chromatography  
3 (HPLC). The mixtures were composed as follows: ‘green’ – *Scenedesmus* sp.,  
4 *Chlamydomonas* sp., *Monoraphidium* sp., *Chlorella* sp., *Micratinium* sp.; ‘blue’ –  
5 *Mycrocystis* sp., *Synechococcus* sp., *Aphanizomenon* sp., *Anabaena* sp.; ‘brown’ – *Cyclotella*  
6 sp., *Nitzschia* sp., *Synedra* sp., *Ceratium* sp., *Peridinium* sp.; ‘mixed’ – *Cryptomonas* sp.  
7 FluoroProbe can measure algal concentrations up to 200  $\mu\text{g chl-}a\ \text{l}^{-1}$  with a nominal resolution  
8 of 0.05 chl-*a*  $\mu\text{g l}^{-1}$ . FluoroProbe data were found highly correlated with cell counts and  
9 spectrophotometric total chl-*a* measurements (see Leboulanger et al., 2002; Gregor et al.,  
10 2005), indicating the reliability of the instrument. In this study, community composition refers  
11 to the spectral algal classes based on the FluoroProbe system rather than the traditional  
12 classification.

13

14 The phytoplankton composition and concentration at Shiraho Reef were quantified before and  
15 after the typhoon, which occurred on August 4-5, 2005. FluoroProbe profile measurements  
16 were performed at stations R1 to R12 (Fig. 1) simultaneous with water sampling and STD  
17 profile measurements. The first measurement was conducted on July 26, 2005. After the  
18 typhoon, measurements were carried out on three occasions (Aug 6, 8, and 11, 2005) to  
19 determine how phytoplankton responded to changes in water quality, particularly nutrient  
20 concentrations. The FluoroProbe was also deployed at some reef stations (i.e., R1, R9, R12)  
21 to obtain one-day continuous measurements at each station to investigate the diurnal  
22 variations in microalgal concentration and composition. The instrument, attached to a buoy,  
23 was submerged to a depth of about 1 meter and recorded data at one-minute intervals.

24

25 In summer 2006, phytoplankton concentrations were measured again with the objective of  
26 assessing differences in microalgae composition and concentration between inner reef and

1 outer reef areas (20-40 meters deep). Profiles of algal concentrations in the water column  
2 were obtained at various points in the inner and outer reef areas before and after several  
3 rainfall events. The first measurements at the outer reef and inner reef were respectively  
4 conducted on August 16 and August 17, 2006, days after several minor rainfall events. On  
5 August 23, 2006, stronger rains occurred and several days after this event, the second set of  
6 measurements at the inner and outer reefs was conducted on August 29 and August 30,  
7 respectively.

8

### 9 *2.2.3 Assessment of groundwater discharge zones*

10 In order to determine the potential influence of groundwater on different locations of the reef,  
11 discharge zones were identified. Groundwater discharge zones were assessed using a method  
12 similar to that employed by Umezawa et al. (2002a). During low tide conditions on August 18,  
13 2006, two sets of samples were taken at nearshore reef stations TN1 to TN9 and TS1 to TS7  
14 (Fig. 1), one at about 5 meters from the low water line and the other at around 20 meters. This  
15 was done to evaluate nutrient concentration differences at points near and far from the low  
16 water line and to determine if groundwater discharge was affecting nutrient concentrations.  
17 To achieve this, other water parameters were evaluated as well. An STD-type instrument  
18 (AAQ-1183 by Alec Instrument Co., Japan) was used to measure the following parameters:  
19 turbidity, chl-*a*, dissolved oxygen, pH, water temperature, salinity, and conductivity;  
20 salinity/conductivity would be the most useful parameters in testing for the discharge of  
21 groundwater.

22

### 23 *2.2.4 Measurement of benthic microalgae*

24 Benthic microalgal measurements were conducted during low tides at the Todoroki River  
25 mouth area and along Shiraho coast. Benthic microalgal concentrations were measured in situ  
26 using bbe Benthofluor (bbe Moldaenke). Benthofluor is a benthic fluorometer for in-situ

1 qualitative and quantitative assessment of benthic microalgae populations (Aberle et al.,  
2 2006). This instrument utilizes the fluorometric characteristics of different algal pigments and  
3 enables a rapid evaluation of the community structure and distribution of microalgae at high  
4 spatial and temporal resolutions, making it ideal for large-scale assessment of spatial and  
5 temporal variations of algal populations in sediments. Benthofluor can differentiate benthic  
6 microalgae into diatoms, and green and bluegreen microalgae. A detailed set of measurements  
7 was conducted in the vicinity of the Todoroki River mouth on August 26-27, 2006 in order to  
8 determine the spatial distribution of benthic microalgae in this area and to gain insight into the  
9 factors that might be affecting microalgal abundance and distribution. The nutrient  
10 concentration in this area can be considered relatively high compared to other parts of Shiraho  
11 Reef. Benthic microalgae along the stretch of Shiraho coast were then measured from August  
12 28 to September 1, 2006 to determine their spatial distribution in relation to nutrient  
13 concentrations as influenced by groundwater discharge. No rains occurred during this period;  
14 hence, there were no discharges from the agricultural drainage outlets. At each Benthofluor  
15 station (i.e., TN1-TN9; TS1-TS7) in Fig. 1, three locations (i.e., small areas) were chosen and  
16 for each location, benthic algal concentrations were measured at three points. To increase  
17 comparability, measurement points were mostly chosen on sandy substrates. The final value  
18 for a station was then computed by averaging the means of the three locations. At each station,  
19 water samples for subsequent nutrient analysis were taken at a distance of about 1 meter from  
20 the low water line.

21

#### 22 2.2.5 Data Analysis

23 Field measurement and monitoring data were analyzed using graphical and statistical  
24 techniques. The data were organized and visualized in a geographic information system  
25 (ArcGIS™ 9, ESRI Inc.) to see the spatial distribution of variables measured and the  
26 relationships between them across geographical space. Relationships between two variables

1 were examined using bivariate correlation analysis with significance testing. Multiple factor  
2 analysis (MFA; see Escofier and Pages, 1994) was used to investigate in more detail the  
3 relative influence of nutrient supply and availability/limitation on phytoplankton  
4 concentration and class composition using data from summer 2005. MFA was utilized for  
5 simultaneous analysis of several tables (groups/sets) of variables. Using this method, common  
6 structures present in all or some of the sets of variables can be found. MFA makes use of  
7 successive application of principal components analysis (PCA). PCA is first applied to each  
8 data set. Each data set is then normalized. The normalized data sets are then merged to form a  
9 unique matrix on which a global PCA is applied (Abdi and Valentin, 2007). MFA was  
10 performed using XLSTAT (an MS Excel add-in). Three tables were used: phytoplankton  
11 concentration/composition, nutrient concentration, and nutrient (molar) ratios.

12

### 13 **3. Results**

#### 14 ***3.1 Terrestrial influence on turbidity and chl-a of the reef***

15 The salinity at most stations exhibited several drops before the typhoon on August 4-5, 2005  
16 (Fig. 2) due to river and groundwater discharge. These resulted in minor increases in turbidity.  
17 The pulses of freshwater gradually increased pre-typhoon levels of chl-a, particularly at  
18 stations R3, R5, R7, R9, and R10. Salinity declines were minimal at channel stations R1 and  
19 R6. At these two stations, increases in chl-a were smaller and more gradual. However,  
20 turbidity in the reef increased drastically as a result of increased sediment discharge from the  
21 Todoroki River and sediment resuspension due to the typhoon. The salinity after the typhoon  
22 dropped dramatically, particularly at R8, the closest station north of the Todoroki River  
23 mouth. Consequently, chl-a levels at most stations (except R5 and R7) increased beyond pre-  
24 typhoon levels as described by Yamamoto et al. (2006).

25

26

Fig. 2

1

### 2 ***3.2 Spatial distribution of nutrients in the inner reef***

3 In general, no appreciable differences in nutrient concentrations between near-surface and  
4 near-bottom samples in the inner reef were observed before and after the typhoon. Prior to the  
5 typhoon, low levels of nutrients were observed in general. However, relatively higher  
6 concentrations were found at stations R12 for almost all forms of dissolved nutrients (Fig. 3).  
7 Nitrate concentrations were relatively high at stations located near agricultural fields,  
8 particularly R5 and R7. After the typhoon, the spatial distribution significantly changed with  
9  $\text{PO}_4^{3-}\text{P}$ ,  $\text{SiO}_2\text{-Si}$  and  $\text{NO}_3^-\text{-N}$  concentrations generally increasing. The highest concentrations  
10 and rates of increase for these nutrients occurred at stations R5, R7, and R8, located  
11 immediately north of the Todoroki River's mouth.  $\text{NO}_3^-\text{-N}$  and  $\text{SiO}_2\text{-Si}$  concentrations also  
12 increased significantly at R9 and R10. Increased nutrient concentrations were also seen in  
13 Tooru-guchi channel, specifically  $\text{NO}_3^-\text{-N}$ ,  $\text{PO}_4^{3-}\text{P}$  and  $\text{SiO}_2\text{-Si}$ , due to Tooru River discharge.  
14 However, the increases were minimal compared to those observed at the stations close to the  
15 Todoroki River discharge area. At station R3, only minimal changes in nutrient  
16 concentrations were observed. Interestingly,  $\text{NH}_4^+\text{-N}$  concentrations did not exhibit  
17 considerable change before and after typhoon.

18

19

Fig. 3

20

### 21 ***3.3 Distribution of planktonic microalgae in the inner reef (Summer 2005)***

22 FluoroProbe-measured total chl-*a* correlated well ( $r=0.87$ ,  $\rho<0.05$ ) with chl-*a* concentrations  
23 measured using laboratory methods. Before the typhoon, there was a clear distinction in the  
24 distribution of phytoplankton communities between the northern reef stations and the  
25 southern reef stations (Fig. 4a). The phytoplankton community composition at most stations  
26 north of the Todoroki River mouth (with the exception of R8) was characterized by the

1 dominance of diatoms and green microalgae on July 26, 2005. In contrast, bluegreen  
2 microalgae dominated at stations south of the river mouth. Relatively higher contributions of  
3 cryptophyta were also observed at these stations. Increased nutrients in the reef due to river  
4 discharge after the typhoon resulted in an overall increase in total chl-*a* level (as much as 4-  
5 fold) and also a noticeable increase in bluegreen and cryptophyta concentrations, particularly  
6 in areas where diatoms and green algae previously dominated (north of the Todoroki River  
7 mouth) (Fig. 4b, 4c). At these areas, increased nutrient levels, notably nitrate and phosphate,  
8 were observed. Considerable increases in green microalgae and diatom contribution were  
9 observed in reef waters south of the Todoroki River mouth. The proportion of bluegreen  
10 microalgae increased further a few days later. On August 11, bluegreen microalgae accounted  
11 for as much as 80% of the total chl-*a* (Fig. 4d).

12  
13 Fig. 4

### 15 ***3.4 Planktonic microalgae in the inner and outer reef (Summer 2006)***

16 On August 16-17, 2006, depth-averaged chl-*a* levels (FluoroProbe-measured) were notably  
17 higher in the outer reef compared to those in the inner reef (Fig. 4e) with exceptions at two  
18 stations – one near the Todoroki River mouth and the other near one of the drainage outlets.  
19 In the upper 2 meters of the water column, chl-*a* in the outer reef was as low as that in the  
20 inner reef (about 0.1  $\mu\text{g L}^{-1}$ ). Outer reef chl-*a* profiles (Fig. 5) indicated increasing  
21 concentration from less than 0.1  $\mu\text{g L}^{-1}$  (near-surface) to about 0.3–0.4  $\mu\text{g L}^{-1}$  (at maximum  
22 measurement depth of 15–20 meters). The vertical distribution of phytoplankton exhibited  
23 some degree of dependence on water density ( $\sigma_t$ ), with higher phytoplankton biomass at  
24 deeper layers of higher  $\sigma_t$ . Phytoplankton community compositions in the inner reef and outer  
25 reef were notably different. Inner reef microalgal compositions varied greatly from station to  
26 station, with three to four microalgal groups present at each. At outer reef stations, diatoms

1 (the most abundant) and green microalgae were dominant and the percentage contributions  
2 from bluegreen microalgae and cryptophytes were negligible. A major rainfall event occurred  
3 on August 23, considerably increasing river discharge. Total chl-*a* in the inner reef increased  
4 significantly at most stations based on the August 29 measurements. Depth-averaged chl-*a*  
5 concentrations in the inner reef became comparable or even higher than those in the outer reef.  
6 The phytoplankton community composition changed as well. Diatoms became dominant in  
7 the northern part of the inner reef. Green microalgae and diatoms dominated in areas near the  
8 Todoroki River mouth. On the other hand, the composition in the outer reef remained almost  
9 unchanged though the chl-*a* concentrations changed. The water column became more  
10 stratified, resulting in a more defined gradation of phytoplankton biomass, especially diatoms.  
11 For both measurement periods, diatoms and green microalgae dominated outside the reef with  
12 depth-averaged concentrations in the ranges of 0.037-0.233  $\mu\text{g L}^{-1}$  and 0.019-0.146  $\mu\text{g L}^{-1}$ ,  
13 respectively, while bluegreen microalgae and cryptophytes were at near-zero levels.

14  
15 Fig. 5

### 16 17 ***3.5 Diurnal variation of planktonic microalgae***

18 Data from the one-day deployments of FluoroProbe at stations R1, R9, and R12 revealed  
19 dynamic diurnal variations in chl-*a* concentration and phytoplankton community composition  
20 in the inner reef (Fig. 6). Measurements at R12 were made before the typhoon while at R1  
21 and R9; measurements were made after the typhoon. Since nutrients were not measured or  
22 monitored at these sample times, only the influence of other variables (e.g., turbidity, solar  
23 radiation) is presented. The overall level of total chl-*a* concentration was highest at R9, which  
24 was located to the immediate south of the Todoroki River mouth. At station R1, the total chl-*a*  
25 level was higher on August 11, 2005 compared to levels the following day, possibly due to  
26 higher solar radiation in the previous day. Chl-*a* levels peaked a few hours after the maximum

1 solar radiation and gradually declined during the night. Diatoms started to dominate during  
2 low tide from around 15:30 on August 11. The wind velocity was relatively high throughout  
3 the R1 monitoring period at about  $8 \text{ m s}^{-1}$ . During the night, increased concentrations of  
4 cryptophytes and cyanobacteria were detected. Green microalgae were only present during the  
5 day. At station R9, the relatively high turbidity generally increased during the monitoring  
6 period due to the Todoroki River plume. Initially, cryptophytes and diatoms dominated  
7 starting from around 5 PM. Cryptophytes were predominant when light levels were low and  
8 turbidity was high. These were replaced by cyanobacteria, which were evidently dominant  
9 during the night and also during the following day when turbidity increased due to river  
10 plume. Cyanobacteria reached  $0.8 \mu\text{g L}^{-1}$  after 17:00 on August 15. The concentration and  
11 percentage contribution of cryptophytes and diatoms were higher on August 14 than on  
12 August 15. Wind speed was relatively higher on August 14. Similarly, peak chl-a levels at  
13 station R12 lagged behind the peak solar radiation levels. Diatoms dominated at this station.  
14 Green microalgae were present only during the day. Cyanobacteria were again detected  
15 during the night with concentrations much lower than those observed at R1 and R12.

16

17

Fig. 6

18

### 19 ***3.6 Exploratory statistical analysis of phytoplankton and nutrient relations***

20 Multiple factor analysis (MFA) biplots (Fig. 7) indicate simultaneously all the variables and  
21 their corresponding factor loadings (i.e., correlations of a variable with a factor). If two  
22 variables are highly correlated, they would have similar degrees of correlation with the factors  
23 that would translate in a biplot as two lines close to each other and of comparable length.  
24 MFA results indicated that the first two factors (F1, F2) accounted for 63.23% to 73.22% of  
25 the total variability in the data set.

26

1 For microalgal concentrations on July 26, 2005 (pre-typhoon), diatoms were closely  
2 associated with the nutrient ratios  $\text{SiO}_2/\text{PO}_4^{3-}$ ,  $\text{SiO}_2/\text{DIN}$ , and  $\text{NH}_4^+/\text{PO}_4^{3-}$  (Fig. 7a). DIN is  
3 dissolved inorganic nitrogen ( $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and  $\text{NH}_4^+$ -N).  $\text{SiO}_2$  concentration per se  
4 seemed to be less important than  $\text{SiO}_2/\text{PO}_4^{3-}$  and  $\text{SiO}_2/\text{DIN}$  for diatoms. Bluegreen microalgae  
5 and cryptophyta were closely associated with each other. However, no clear association with  
6 specific nutrient levels and ratios could be inferred. On August 8, 2005, bluegreen and green  
7 microalgae had similar distributions, and were associated with most nutrients (i.e.,  $\text{NO}_3^-$ ,  
8  $\text{PO}_4^{3-}$ ,  $\text{SiO}_2$ , DIN) and to the nutrient ratios  $\text{SiO}_2/\text{NH}_4^+$  and  $\text{NO}_3^-/\text{PO}_4^{3-}$ . Bluegreen algae  
9 exhibited negative relationships with  $\text{SiO}_2/\text{DIN}$ ,  $\text{SiO}_2/\text{NO}_3^-$ , and  $\text{NH}_4^+/\text{PO}_4^{3-}$  on both  
10 occasions. Correlations of total chl-*a* and green microalgae with F1 were similar to that of  
11  $\text{NO}_3^-/\text{PO}_4^{3-}$  on both occasions. The interplay among the variables resulted in the clustering of  
12 similar stations. On July 26, 2005, most stations, except R3 and R12, formed a cluster in the  
13 factor space (Fig. 7a). On August 8, 2005, R7 and R8, the stations to the immediate north of  
14 Todoroki River mouth, formed a separate cluster.

15  
16 The percentage of bluegreen microalgae and cryptophyta seemed to have similar distribution  
17 for most stations on July 26, 2005 (Figs. 4a, 7b). The same can be said for the percentages of  
18 diatoms and green microalgae. However, %Bluegreens was inversely related (diametrically  
19 opposite) to %Diatoms just as %Cryptophyta was to %Green. In general, this implies that  
20 stations with relatively high percentages of bluegreen (/green) microalgae were likely to have  
21 relatively low percentages of diatoms (/cryptophyta). %Bluegreen and %Cryptophyta were  
22 associated mainly with  $\text{PO}_4^{3-}$  concentration. %Diatoms and %Green were related to  
23  $\text{NH}_4^+/\text{PO}_4^{3-}$  primarily and also to  $\text{SiO}_2/\text{DIN}$ ,  $\text{SiO}_2/\text{NO}_3^-$ , and  $\text{SiO}_2/\text{PO}_4^{3-}$ . On August 8, 2005,  
24 %Bluegreens showed a closer association with  $\text{NO}_3^-/\text{PO}_4^{3-}$ . Note again that %Diatoms and  
25 %Cryptophyta were diametrically opposite to %Bluegreen and to %Green, respectively. The  
26 clustering of stations on July 26 was similar to previous observations, with R3 and R12 being

1 distinctly different from the other stations. On August 8, most stations formed a cluster with  
2 the exclusion of R5, R7, R8, and R10.

3

4

Fig. 7

5

### 6 ***3.7 Distribution of CDOM in reef waters***

7 On July 26, 2005 (before the typhoon), the highest concentration of CDOM was observed  
8 close to the Todoroki River mouth (Fig. 8). After the passage of the typhoon, the relative  
9 CDOM levels generally increased throughout the reef (particularly in areas north of the  
10 Todoroki River mouth), partly due to runoff from the Todoroki River. Based on  
11 measurements in the Todoroki River, the CDOM concentrations of river waters were at least  
12 four times higher than CDOM in reef waters. For summer 2006, measurements on August 17  
13 indicated that CDOM concentrations in the inner reef were considerably higher than outer reef  
14 levels. Inner reef CDOM concentrations increased further two weeks later, possibly due to  
15 river runoff caused by rains on August 21-23, 2006. In the outer reef, CDOM was generally  
16 higher in the upper few meters of the water column (Fig. 5).

17

18

Fig. 8

19

20 The time series of CDOM variation at three stations in Shiraho Reef indicates that high  
21 amounts of CDOM are produced within the reef (Fig. 6). CDOM concentrations increased  
22 significantly (as much as 4-fold) during the night. CDOM decreased to low levels towards  
23 daytime, potentially indicating photobleaching effect. Importantly, cyanobacterial  
24 concentration exhibited a strong positive correlation with CDOM concentration during the  
25 night until dawn. Over the period from 09:00. to 06:00, the cyanobacteria-CDOM correlation  
26 values for stations R1, R9, and R12 were 0.78, 0.81, and 0.92, respectively.

1

### 2 **3.8 Into-reef groundwater discharge assessment**

3 Nutrient concentrations of samples taken at a distance of around 5 meters from the low water  
4 line were generally higher than those taken at about 20 meters (Fig. 9a). Among the northern  
5 stations (TN1-TN9), nitrate concentrations (5m and 20m) were relatively high at stations TN9  
6 (relatively closer to the Todoroki River mouth) and TN5. Nitrite (5m) was highest at TN9 and  
7 generally decreased towards TN3. For stations on the southern coast (TS1-TS7),  $\text{NO}_3^-$   
8 concentrations were highest at TS4 and TS5, and comparable to  $\text{NO}_3^-$  levels at TN9.  $\text{NH}_4^+$   
9 peaked at TS3 (5m) while  $\text{NH}_4^+$  (20m) was relatively higher at TS4 and TS5.  $\text{PO}_4^{3-}$   
10 concentrations (5m) were greater at TS3 and TS4 than at any other southern coast station.

11

12 The salinity was generally lower for water samples collected closer (i.e., at 5m) to the low  
13 water line than for samples collected farther away (i.e., at 20m) (Fig. 9b). Groundwater  
14 seepage was visible at several sampling and measurement locations along the beach. The  
15 lower salinity values at station TN5, TS5 and other nearby stations indicated that these were  
16 zones of relatively high groundwater discharge. The salinity correlated strongly and  
17 significantly ( $p < 0.05$ ) with  $\text{NO}_3^-$  concentrations with  $r = -0.87$  and  $r = -0.88$  for stations TN1-  
18 TN9 and TS1-TS7, respectively.

19

20

Fig. 9

21

### 22 **3.9 Distribution of benthic microalgae**

23 The river mouth area was dominated by bluegreen algae (most abundant) and diatoms (Fig.  
24 10b). Similar high magnitudes of bluegreen algae were found on the coast adjacent to  
25 agricultural fields north of the river mouth and at the southernmost part of Shiraho reef,  
26 relatively close to residential areas (Fig. 10a). Benthic green microalgae were much lower in

1 concentration than cyanobacteria or diatoms. They were absent or undetectable along some  
2 parts of the coast. Even in the vicinity of the Todoroki River mouth, where green macroalgae  
3 were abundant during the measurement period, the concentrations of green microalgae were  
4 less than  $0.75 \mu\text{g cm}^{-2}$ . In contrast, the concentrations of cyanobacteria and diatoms were  
5 higher at parts of the river mouth where green macroalgae were observed.

6

7

Fig. 10

8

9 For the north coast stations (TN1-TN9), diatoms were positively related to  $\text{PO}_4^{3-}\text{-P}$  and also to  
10  $\text{NH}_4^+\text{-N}$  (not shown) to some extent (Fig. 11). Bluegreen microalgae appeared to be  
11 influenced by  $\text{PO}_4^{3-}\text{-P}$  as well. The spatial variation for  $\text{NO}_3^-\text{-N}$  very much resembled that of  
12 diatoms. For stations TS1-TS7, the distribution of diatoms corresponded strongly and  
13 significantly with  $\text{PO}_4^{3-}\text{-P}$  concentrations ( $r = 0.81$ ,  $p < 0.05$ ). The spatial variations of diatoms  
14 and  $\text{NH}_4^+\text{-N}$  (not shown) for TS1-TS7 were also found similar. Considering all station, the  
15 overall trends of bluegreen algae, diatoms, DIN, and phosphate were similar to a certain  
16 extent.

17

18

Fig. 11

19

## 20 **4. Discussion**

### 21 ***4.1 Reef Nutrients and the Phytoplankton Response***

22 The similarity of pre-typhoon and post-typhoon spatial distribution of  $\text{NH}_4^+\text{-N}$  concentrations  
23 indicated that  $\text{NH}_4^+\text{-N}$  is mainly produced within the reef. Indeed,  $\text{NH}_4^+\text{-N}$  is emitted from  
24 sediments of seagrass beds, especially in summer (Miyajima, 2001). For the other nutrients,  
25 the observed spatial distributions may be largely influenced by reef currents, as they are in  
26 agreement with the patterns of current described earlier [see Nadaoka et al. (2001) and

1 Tamura et al. (2007) for detailed description]. Nutrients at R7 and R8 were greatly increased  
2 by northward currents that transported nutrients discharged from the Todoroki River. As  
3 shown by the MFA results, these stations were significantly different from other stations (Fig.  
4 7b). At channel stations R4 and R5, where water exchange between reef and open sea is  
5 relatively great, nutrient concentrations were very low, with minimal change observed  
6 between pre-typhoon and post-typhoon nutrient levels. The strong current converging on the  
7 Tooru-guchi channel limited the influence of nutrient discharge from the Tooru River on the  
8 reef surrounding Tooru-guchi. Due to this pattern of currents, most of the sediments and  
9 nutrients from the Tooru River were directly flushed to the outer sea via the channel. This  
10 pattern was also responsible for the nutrient concentration at station R3, which remained low  
11 even after the typhoon. These observations indicate that much of the nutrients (except  $\text{NH}_4^+\text{-N}$ )  
12 in the reef came from the Torodoki River and were eventually transported and dispersed to  
13 the north by currents, especially during typhoons and strong rainfall events. Thus, advection  
14 and diffusion of river plume in the reef, and hence nutrients, are strongly governed by the  
15 relative locations of river mouths and channels, especially large channels like Tooru-guchi.

16

17 The increased nutrient levels in the reef elicited varied responses by microalgal communities  
18 at different locations. This is indicative of the complex relationship between nutrient  
19 availability and phytoplankton responses and also reflects the influence of hydrodynamics.  
20 The observed difference in phytoplankton composition (and the resulting change in class  
21 contribution) between reef waters north and south of the Todoroki River mouth can be  
22 attributed to the influence of reef hydrodynamics and nutrient source. The reef area north of  
23 the Todoroki River mouth is subjected to relatively greater influence by terrestrial runoff via  
24 discharge from the Todoroki River and also from agricultural drainage outlets near the coast.  
25 On the other hand, the southern reef area can be considered more influenced by groundwater  
26 discharge from the adjacent lands. The early dominance of green microalgae, cryptophytes,

1 and diatoms (to a certain extent) in the nutrient-enriched reef waters after the typhoon was  
2 possibly due to the relatively efficient growth rates and enhanced uptake rates of these  
3 microalgal groups (Paerl et al. 2003; Delesalle et al. 1993). Green microalgae and  
4 cryptophytes are common in freshwater and brackish water rather than seawater.  
5 Consequently, the dominance of these microalgal classes at some reef stations after the  
6 typhoon may be thought as a result of enhanced freshwater input rather than higher nutrient  
7 availability. However, FluoroProbe measurements in the outer reef pointed to the presence of  
8 marine green microalgae. In the inner reef, cryptophytes were detected (and contributed  
9 significantly) even during low river discharge periods (Fig. 4a, 4e, 4f). FluoroProbe  
10 measurements made in Todoroki River indicated that cryptophytes is not a dominant algae  
11 class in the river. In addition, cryptophyte contribution to total chl-a in nearshore marine  
12 waters elsewhere was found to increase as well at low nutrient concentrations and high  
13 salinities (Tamigneaux et al., 1995). Further investigation through microscopic examination  
14 and phytoplankton pigment analysis by high-performance liquid chromatography (HPLC) is  
15 suggested.

16

17 The observed shift to cyanobacteria dominance may have been aided by grazing pressure (by  
18 zooplankton) as green microalgae are often more palatable than cyanobacteria. Silicon (Si)  
19 limitation may have also occurred, resulting in a decrease in diatom concentration and  
20 contributing to increases in the concentration and contribution of bluegreen algae (Rocha et  
21 al., 2002). This is possible as the increase in Si and P due to typhoon would have induced the  
22 growth of diatoms. As diatoms multiply, however, Si is consumed and may eventually  
23 become limiting relative to other nutrients once river flow reverts back to the normal flow  
24 level and groundwater discharge starts to diminish. At this stage, the availability of nutrients  
25 may favor the growth of cyanobacteria. This is supported by the MFA results, suggesting an  
26 inverse relationship of cyanobacteria percentage with  $\text{SiO}_2/\text{DIN}$  and  $\text{SiO}_2/\text{NO}_3^-$  before and

1 after typhoon. On the other hand, diatoms have a relatively high specific gravity compared to  
2 other microalgae, making them better adapted to highly turbulent conditions than calm-water  
3 conditions. The cyanobacteria dominance after the storm may have been a consequence of  
4 decreased turbulent flow that should have led to sedimentation of diatoms, rather than  
5 depletion of Si. However, diatoms were observed to dominate the northern Shiraho reef on  
6 July 26, 2005, several days before the typhoon. MFA results indicated that diatom percentage  
7 was influenced by  $\text{NH}_4^+/\text{PO}_4^{3-}$ . The increase in  $\text{PO}_4^{3-}$  concentrations after the typhoon  
8 drastically reduced  $\text{NH}_4^+/\text{PO}_4^{3-}$  for all northern reef stations. This potentially led to the  
9 significant reduction in diatom percentage at stations R3, R4, R5, R6 and R7. Furthermore, a  
10 few days after the typhoon, diatom dominance was observed only at some reef stations,  
11 particularly those that were located near channels, where turbulence was usually high. It is  
12 important to note that the cryptophyta concentration and percent contribution generally  
13 increased immediately after the typhoon when turbidity was still high compared to calm-water  
14 conditions. This can be explained by the cryptophyte's motility and its ability to thrive in low  
15 light conditions (Bergmann, 2004). However, as light penetration of the water column  
16 increases, cyanobacteria may replace cryptophytes. Thus, proliferation of cyanobacteria  
17 several days after the typhoon was more likely caused by increased nutrient availability  
18 favorable to cyanobacteria and aided by improved light conditions.

19

20 As MFA results indicated, the percentage contribution to total chl-*a* by cyanobacteria is  
21 somehow inversely related to that of diatoms, potentially providing insights into the general  
22 phytoplankton succession in the reef. This was tested using the one-day continuous data  
23 obtained at R1, R9, and R12. Analysis of daytime (i.e., 7AM-5PM) data showed that the  
24 correlation between %cyanobacteria and %diatoms was significantly higher than other pairs  
25 with values of -0.56 (R1, Aug 11, 2005), -0.48 (R9, Aug 15, 2005) and -0.66 (R12, Aug 2,  
26 2005). The correlations are not that strong as phytoplankton community composition is highly

1 dynamic and subject to several complex interacting factors. Nonetheless, an inverse  
2 relationship exists between these two algal classes in Shiraho Reef, which requires further  
3 investigation.

4  
5 Shifts in microalgal community structure as a result of alterations in nutrient input may  
6 subsequently affect upper trophic levels (Armitage et al. 2006) if such shifts persist. However,  
7 this does not seem to be the case for Shiraho Reef, where increases in nutrient levels in the  
8 reef usually occur only during typhoons and major rainfall events. Shifts in phytoplankton  
9 composition resulting from enhanced nutrient levels in the reef seem to be temporary.  
10 Nonetheless, the evident change in dominance of green microalgae and diatoms to dominance  
11 by bluegreen microalgae as a result of nutrient enrichment implies that microalgae can be  
12 used as indicators of coastal eutrophication.

13

#### 14 ***4.2 Phytoplankton: Inner Reef vis-à-vis Outer Reef***

15 Chl-*a* concentrations in Shiraho Reef can be higher or lower than that of the adjacent open sea.  
16 The low chl-*a* values in the inner reef on August 17 may be attributed primarily to low  
17 nutrient levels since, for about two weeks prior to the measurement day, no major rainfall  
18 event occurred that would have caused much terrestrial runoff to deliver sediments and  
19 nutrients. This low concentration of chl-*a* in the inner reef may also have been caused by  
20 grazing and higher activity of filter feeders inside the reef. In the outer reef, the occurrence of  
21 higher concentrations of chl-*a* at depths of 15 to 25 meters was partly due to good light  
22 penetration since CDOM in the water column was very low. Lower chl-*a* levels near the water  
23 surface indicate photoinhibition by phytoplankton.

24

25 Only a few studies have investigated the differences in phytoplankton community  
26 composition between coral reefs and the adjacent open sea. In Shiraho Reef, the four

1 'spectral' classes of microalgae were present. The near absence of cryptophytes in the outer  
2 reef can be due to extremely low organic matter (Fig. 11) in this area as cryptophytes are  
3 principally prevalent in areas with high concentrations of organic matter (Bergmann, 2004).  
4 Cryptophytes are able to maximize light absorption/utilization and capitalize on alternative  
5 fuel sources (e.g., organic nutrients) under low light conditions (Bergmann, 2004), hence the  
6 increased concentration of cryptophytes at low light level and increasing CDOM (Fig. 7).  
7 Cyanobacteria were found in the inner reef only due to the higher nutrient availability as  
8 described earlier. The difference in phytoplankton community compositions in the inner reef  
9 and outer reef may be partly explained by expelled zooxanthellae that occur only in the inner  
10 reef. The foregoing indicates that reef environments significantly influence phytoplankton  
11 composition primarily through nutrient availability and the abundance of organic matter.

12

### 13 ***4.3 Spatial and Temporal Distribution of CDOM in Shiraho Reef***

14 CDOM protects reef organisms by absorbing harmful ultraviolet radiation, which is linked to  
15 coral bleaching (Otis et al. 2004). It is important that the spatial and temporal distribution of  
16 CDOM in the reef as influenced by terrestrial discharge and other factors be assessed. The  
17 observed increase in inner reef CDOM level after increased discharge from the Todoroki  
18 River indicates that river water is one of the primary sources of CDOM in Shiraho Reef.  
19 However, the observed difference between CDOM in river water and in inner reef water is not  
20 large (i.e., only 4-fold). On the other hand, the inner reef CDOM concentration is several  
21 times higher than that in the outer sea (Fig. 11). If CDOM in the inner reef were totally  
22 supplied by the river, the salinity of the inner reef water should be much less than that in the  
23 outer sea. However, this is not the case and CDOM must have come from sources within  
24 Shiraho Reef such as seagrass meadows, macroalgal colonies, and bottom sediments. CDOM  
25 can be derived from benthic habitats such as seagrass meadows (Stabenau et al., 2004). It can  
26 also be produced by heterotrophic bacteria through the alteration of algal-derived dissolved

1 organic matter (Rochelle-Newall and Fisher, 2002). CDOM is produced by the decomposition  
2 of algal and seagrass detritus. The strong positive correlation between CDOM concentration  
3 and cyanobacterial concentration during the night-dawn transition may indicate that CDOM  
4 in the reef is derived from DOM precursors produced by cyanobacteria.

5

#### 6 ***4.4 Groundwater Discharge, Nutrients, and Benthic Microalgae***

7 Nutrient contribution from groundwater discharge into Shiraho Reef is significant. For  
8 southern Shiraho Reef, dissolved inorganic nitrogen loading through groundwater discharge  
9 was estimated to be 7.3-10.4 Mg N/year, accounting for 35% of the total nitrogen input  
10 (Umezawa et al., 2002a). The contribution of nutrients can be expected to increase  
11 dramatically with increased groundwater discharge after relatively long rainfall events or  
12 typhoons. This is particularly important in areas distant from the direct influences of the  
13 Todoroki River discharge. The observed salinity decreases at stations TS4 and TS5 was in  
14 agreement with the observations of Kawahata et al. (2000). The nearshore salinity plots (Fig.  
15 12b) indicates the influence of subsurface geology on groundwater discharge. Greater  
16 groundwater contribution was observed at the coast near areas dominated by limestone and  
17 conglomerate bedrocks, both with relatively high permeability. On the other hand, the  
18 presence of relatively impermeable metamorphic bedrock close to the coast potentially limits  
19 groundwater seepage. Generally, higher concentrations of nutrients were associated with  
20 lower salinity, an indication that nutrient concentrations are influenced by groundwater  
21 discharge, particularly in the nearshore areas of Shiraho Reef.

22

23 The impact of nutrients derived from groundwater discharge on the reef is reflected in the  
24 spatial distribution of benthic microalgae along the Shiraho coast. In general, significant  
25 correlations and trend similarities were found between nutrient concentrations and benthic  
26 microalgae. Apart from the river mouth area, concentrations of both quantities were high in

1 groundwater discharge zones previously identified. As there was no rainfall and subsequent  
2 discharge from the agricultural drainage outlets during the benthic microalgal measurement  
3 period, the high nutrient concentrations (concurrent with low salinity) measured during low  
4 tide can be attributed primarily to groundwater discharge. Benthic diatoms correlated well  
5 with the  $\text{NO}_3^-$  concentrations of water samples taken during low tides. However, the  
6 groundwater in the study area is also known to contain high concentrations of  $\text{SiO}_2\text{-Si}$ ,  
7 ranging from 100-200  $\mu\text{M}$  (Kawahata et al., 2000; Miyajima et al., 2007b). This, in addition  
8 to  $\text{NO}_3^-$ , may have benefited benthic diatoms inhabiting groundwater seepage areas. Nutrients  
9 may come from benthic habitats as well (e.g.,  $\text{NH}_4^+$  from seagrass bed sediments), thus  
10 increasing nutrient concentrations independently of groundwater. Nonetheless, benthic  
11 microalgae can be used for assessing the influence of groundwater seepage on the nearshore  
12 reef areas.

13  
14 Nutrients delivered through groundwater discharge may not significantly affect coral reef  
15 biota and habitats located far from the shoreline (Umezawa et al., 2002b). Considering that  
16 most parts of Shiraho Reef are shallow and waters are relatively clear during most of the year,  
17 light penetration to the bottom can be considered sufficient to support benthic microalgae  
18 production. The productive biomass of microphytobenthos (15.0–36.4 mg Chl-*a*  $\text{m}^{-2}$ ) in the  
19 carbonate sediments of southern Shiraho Reef were two orders of magnitude higher than that  
20 of phytoplankton (0.32 mg Chl-*a*  $\text{m}^{-2}$ ) (Suzumura et al., 2002). Thus, assessing its distribution  
21 throughout the reef is also important to understanding the overall contribution of benthic  
22 microalgae to the productivity of Shiraho Reef. The use of a spectrofluorometer for benthic  
23 microalgal measurement is advantageous as measurements can be performed in situ over a  
24 large area without disturbing the substrate. Cyanophyta (bluegreen) and chlorophyta (green)  
25 are relatively fragile and more adhesive compared to diatoms, potentially hampering their  
26 recovery from sediment particles (Suzumura et al., 2002). Thus, this spectrofluorometric

1 technique may provide a more accurate assessment of bluegreen and green microalgae than  
2 conventional extraction methods.

3

#### 4 *4.5 Utility of Microalgae for Assessing Terrestrial Influence on Coral Reef*

5 In this study, the terrestrial influence on Shiraho Reef was explored and assessed in two  
6 modes – influence through terrestrial runoff and influence through groundwater discharge.

7 Terrestrial influence was assessed through sampling of phytoplankton and benthic microalgae  
8 in addition to nutrients and sediments. We demonstrated that nutrient level and availability  
9 not only control phytoplankton concentration but also influence the community composition.

10 The concentration and composition of phytoplankton in turn provides additional information  
11 on the factors affecting them, such as hydrodynamics and water quality (e.g., turbidity). The  
12 benthic microalgae concentration also corresponded to nutrient levels associated with  
13 groundwater discharge, particularly at the nearshore reef areas. Thus, the utility of microalgae  
14 as indicators of terrestrial influence on coral reef was convincingly demonstrated. In addition,  
15 the approach used in this study has a unique merit in that indicators can be measured in situ  
16 and in real-time. On declining reefs, cyanobacteria are increasingly prominent as they are  
17 adaptive to high nutrient concentrations, strong solar radiation, and higher temperatures  
18 (Hallock, 2005). Monitoring of phytoplankton community composition is thus important.

19

#### 20 **Conclusions**

21 Allochthonous inputs such as nutrients, suspended matter, and freshwater can cause adverse  
22 environmental changes for coral reefs. These changes have been recognized as important  
23 agents in the degradation of coral reefs that may eventually lead to complete replacement of  
24 hermatypic corals with macroalgal beds. In this study, indicators based on microalgal  
25 concentration and pigment composition, as measured by portable spectrofluorometric  
26 instruments, were introduced and tested for use in early diagnosis of coral reef ecosystems.

1 This study demonstrates the potential of this approach in detecting microalgal community  
2 changes triggered by large discharge events (e.g., typhoon), as well as those induced by  
3 chronic seepage of nutrient-rich groundwater. Nutrient enrichment by terrestrial discharge  
4 significantly changed phytoplankton community composition and induced cyanobacteria  
5 dominance of Shiraho Reef. Benthic microalgal concentration and composition along Shiraho  
6 coast manifested the influence of groundwater discharge. Therefore, microalgae are useful  
7 indicators of terrestrial influence on coral reefs.

8  
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20

**21 Figure captions**

22 Fig. 1. The study site: Shiraho Reef in Ishigaki Island (Okinawa, Japan). R1-R12 are stations where  
23 various sensors were deployed and where phytoplankton measurement and water sampling were made.  
24 TN1-TN11 and TS1-TS7 are stations for nearshore water sampling and for benthic microalgae  
25 measurement. Shown also is the Todoroki River and watershed (Image: IKONOS<sup>TM</sup> image taken on 3  
26 August 2004)

27

1 Fig. 2. (a) Meteorological condition and Todoroki River discharge during the study period in Summer  
2 2005. Indicated are the dates during which water quality surveys were conducted in Shiraho Reef. (b)  
3 Time-series of turbidity, Chl-a and salinity at various stations in the reef obtained using deployed  
4 turbidity meters and salinometers.

5  
6 Fig. 3. Spatial distribution of various nutrients in Shiraho Reef before typhoon (26 July 2005) and after  
7 typhoon (8 August 2005).

8  
9 Fig. 4. Spatial distribution of class-differentiated planktonic microalgae in Shiraho Reef in Summer  
10 2005 and Summer 2006. Labeled outer reef points are stations for which vertical profiles are shown in  
11 Figure 5.

12  
13 Fig. 5. Typical class-differentiated phytoplankton profiles in outer Shiraho Reef in Summer 2006. Chl-  
14 a concentration is expressed in  $\mu\text{g L}^{-1}$ . Plots correspond to the labeled stations in Fig. 4. CDOM in  
15 relative amounts (unitless) is also shown. Average water depth (H) at each station is indicated.

16  
17 Fig. 6. Diurnal variations of microalgal concentration, class composition and CDOM (measured using  
18 FluoroProbe) at selected stations (R1, R9, R12) in Shiraho Reef. Corresponding water quality  
19 (measured by turbidity meter and salinometer), tide level and weather data are also shown.

20  
21 Fig. 7. Relationship between phytoplankton and nutrients (concentrations & ratios) in Shiraho Reef  
22 (inner) on 26 July (before typhoon) and on 8 August 2005 (after typhoon): (a) phytoplankton  
23 concentration and nutrient; (b) phytoplankton composition and nutrients. The percentage variability of  
24 the data set represented by the factors F1 and F2 are shown in parenthesis. Colors indicate grouping of  
25 variables.

26  
27 Fig. 8. Spatial distribution of CDOM in Shiraho Reef in Summer 2005 and Summer 2006. All data  
28 taken during low tide.

1 Fig. 9. Nutrient concentrations and salinity measured at 5m and 20m from the low water line for  
2 stations TN1-TN9 and TS1-TS7 on 18 August 2006.

3

4 Fig. 10. Benthic microalgal concentrations (a) along the coast of Shiraho Reef and (b) in the vicinity  
5 of Todoroki River mouth measured using Benthofluor from 26 August to 1 September 2006.

6

7 Fig. 11. Benthic microalgae and nutrient concentrations measured during low tide conditions for  
8 stations TN1-TN9 and stations TS1-TS7 along nearshore Shiraho Reef.

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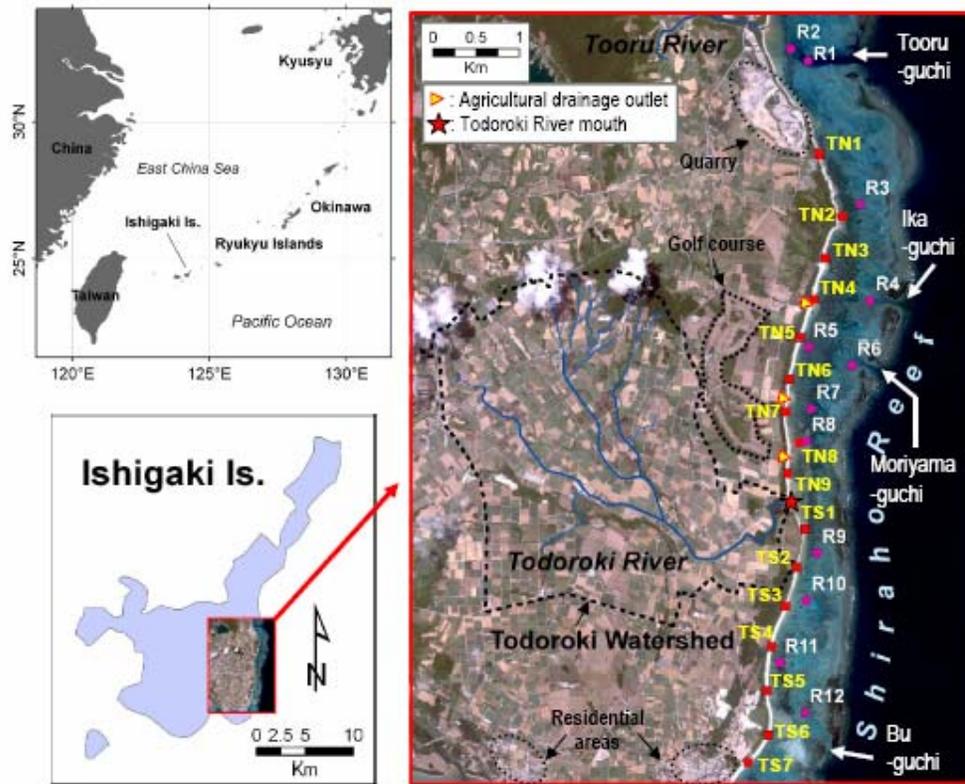


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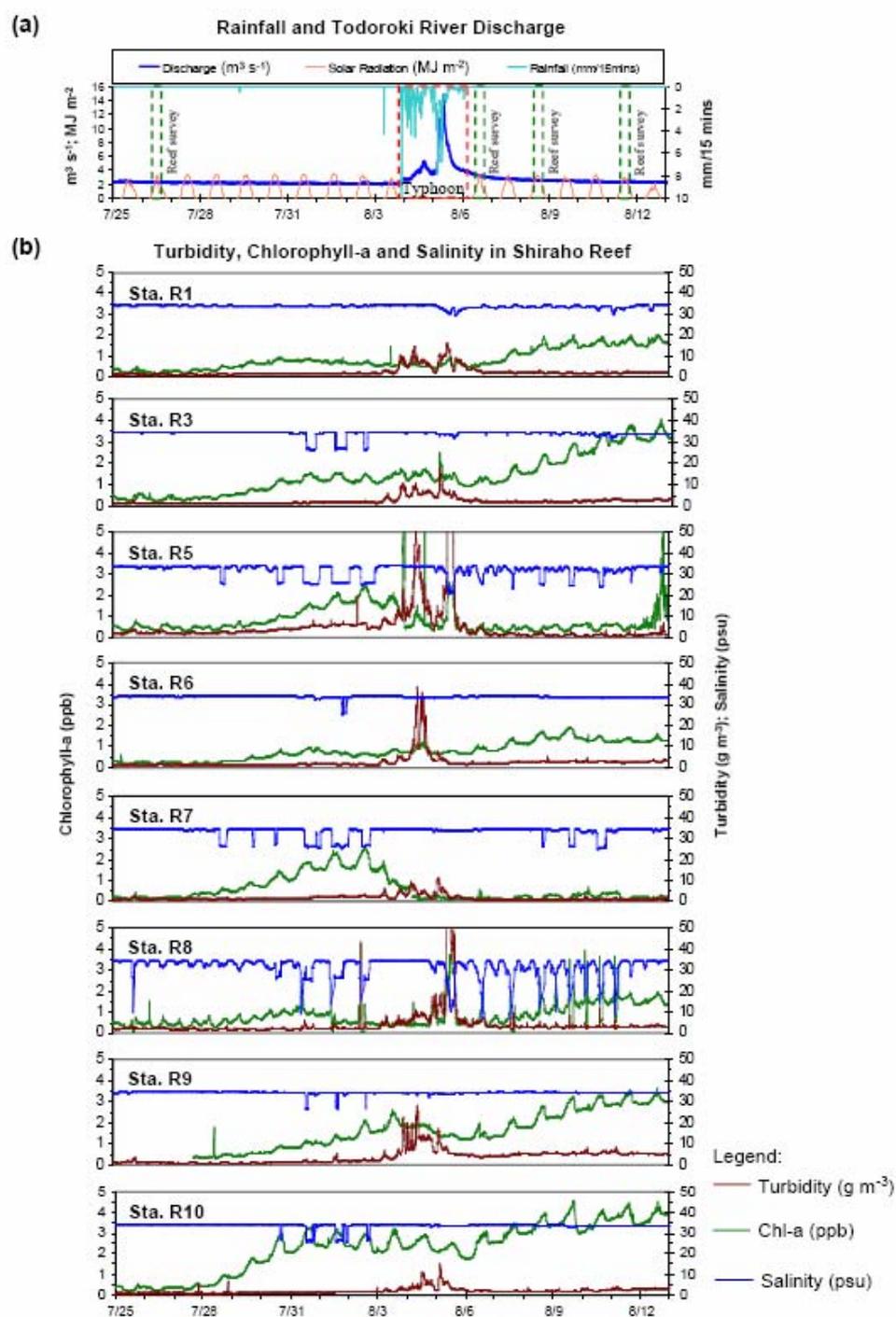


Fig. 2. (a) Meteorological condition and Todoroki River discharge during the study period in Summer 2005. Indicated are the dates during which water quality surveys were conducted in Shiraho Reef. (b) Time-series of turbidity, Chl-a and salinity at various stations in the reef obtained using deployed turbidity meters and salinometers.

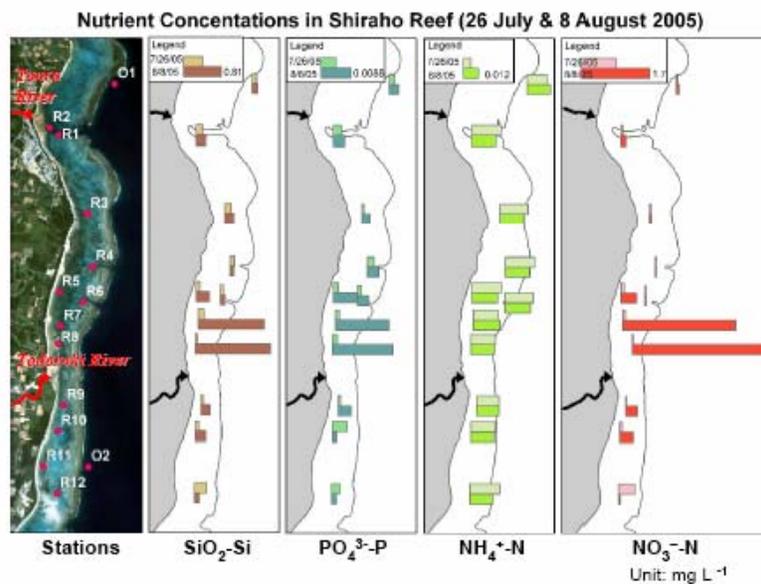


Fig. 3. Spatial distribution of various nutrients in Shiraho Reef before typhoon (26 July 2005) and after typhoon (8 August 2005).

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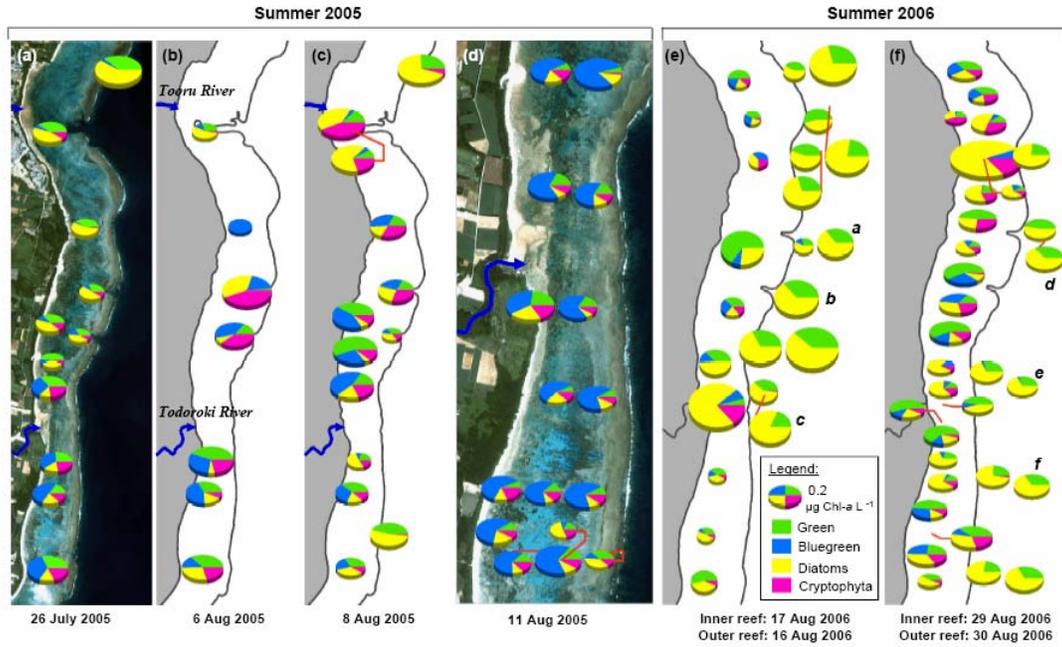


Fig. 4. Spatial distribution of class-differentiated planktonic microalgae in Shiraho Reef in Summer 2005 and Summer 2006. Labeled outer reef points are stations for which vertical profiles are shown in Figure 5.

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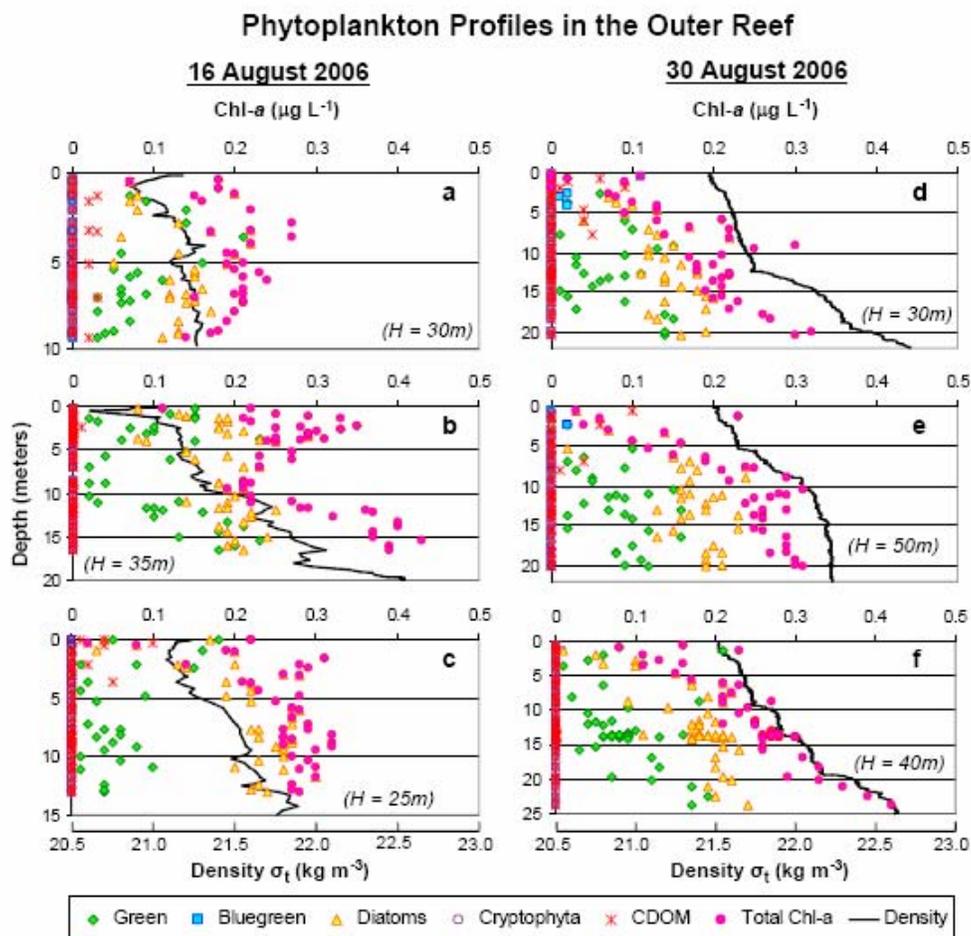


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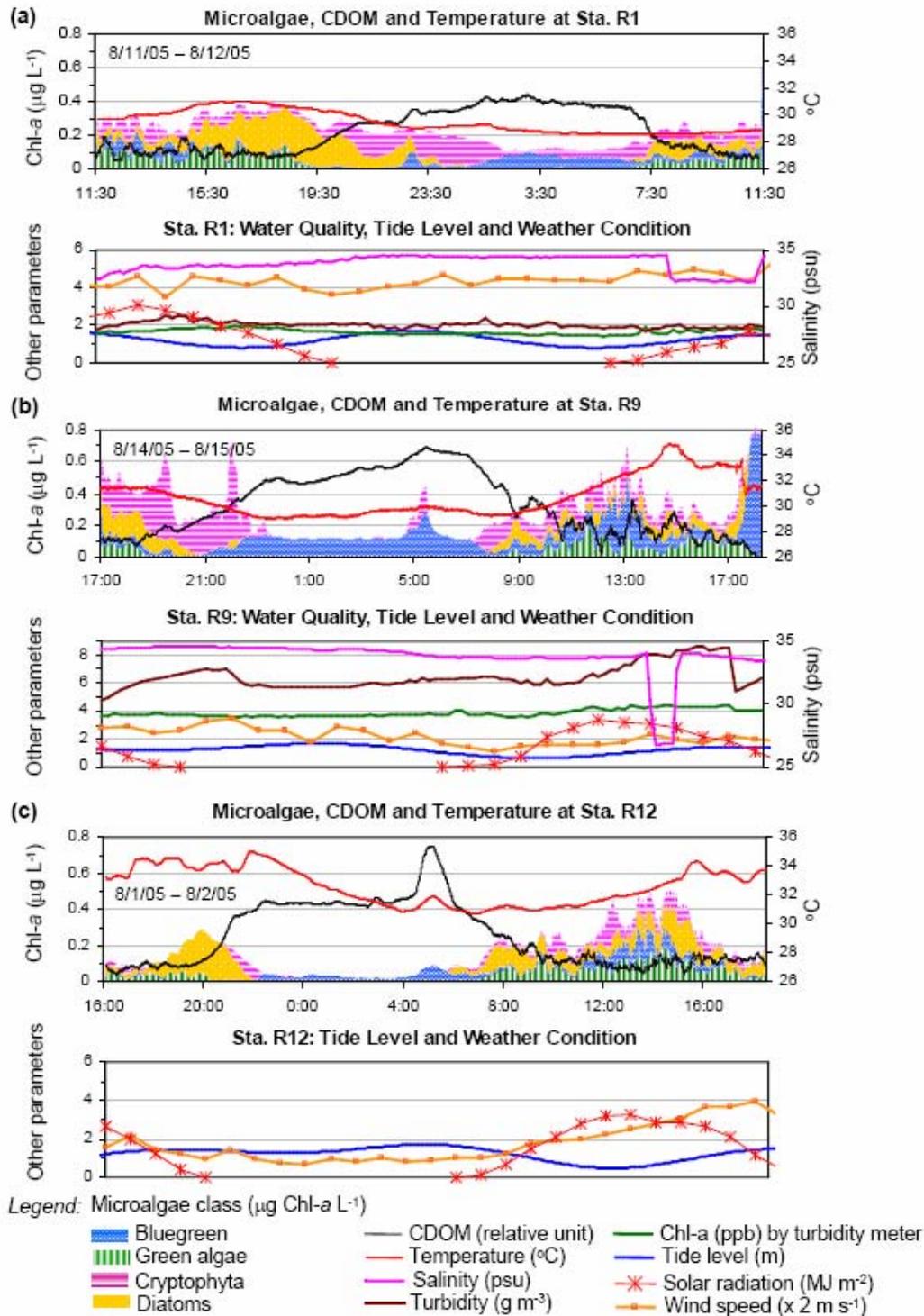
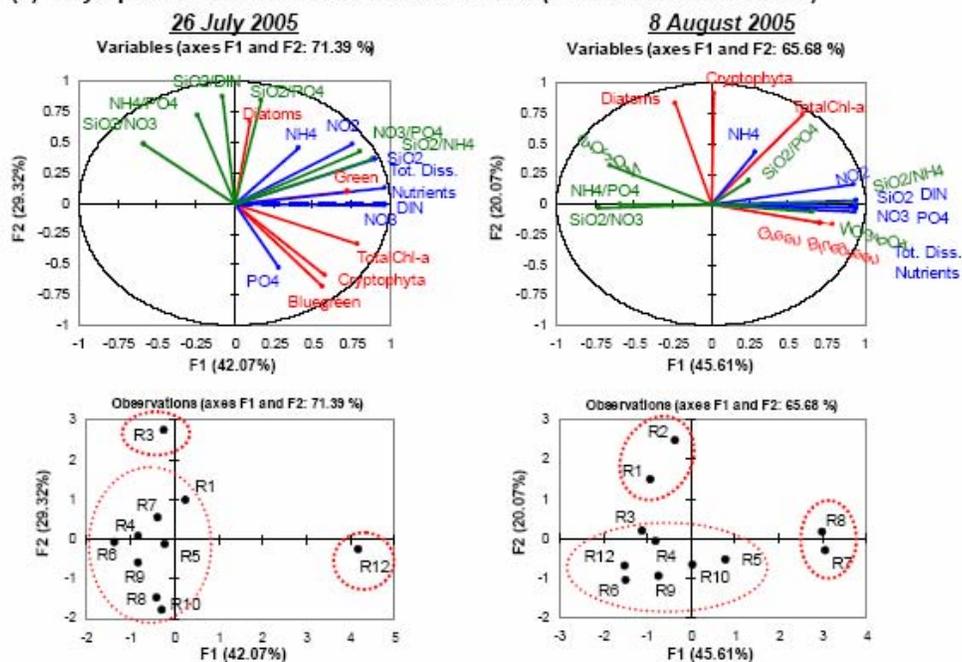


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## (a) Phytoplankton concentration and nutrients (concentration and ratio)



## (b) Phytoplankton composition and nutrients (concentration and ratio)

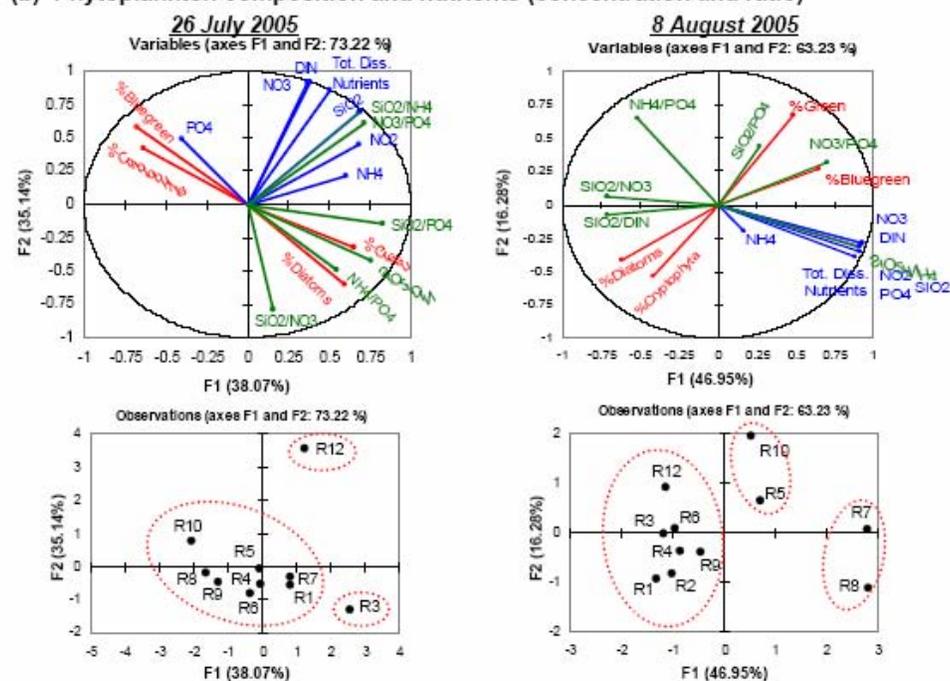


Fig. 7. Relationship between phytoplankton and nutrients (concentrations & ratios) in Shiraho Reef (inner) on 26 July (before typhoon) and on 8 August 2005 (after typhoon): (a) phytoplankton concentration and nutrient; (b) phytoplankton composition and nutrients. The percentage variability of the data set represented by the factors F1 and F2 are shown in parenthesis. Colors indicate grouping of variables.

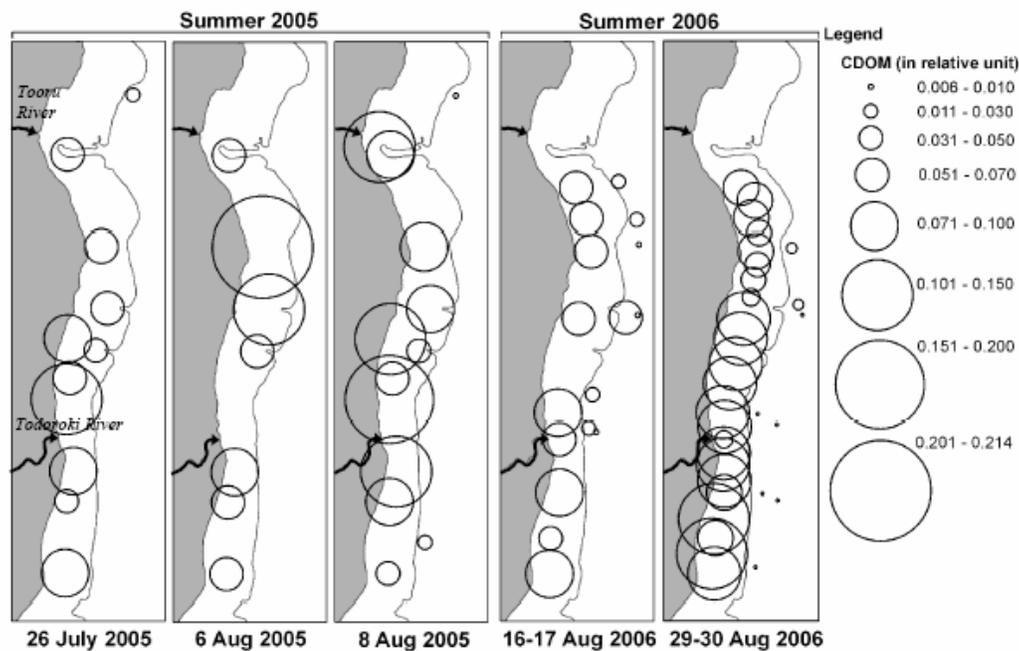


Fig. 8. Spatial distribution of CDOM in Shiraho Reef in Summer 2005 and Summer 2006. All data taken during low tide.

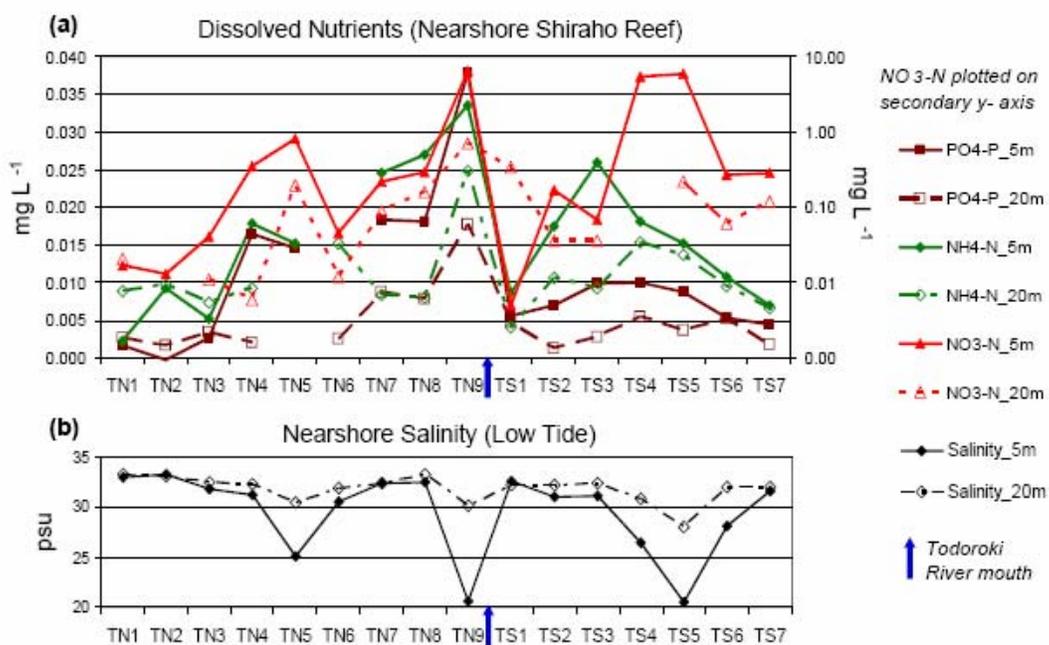


Fig. 9. Nutrient concentrations and salinity measured at 5m and 20m from the low water line for stations TN1-TN9 and TS1-TS7 on 18 August 2006.

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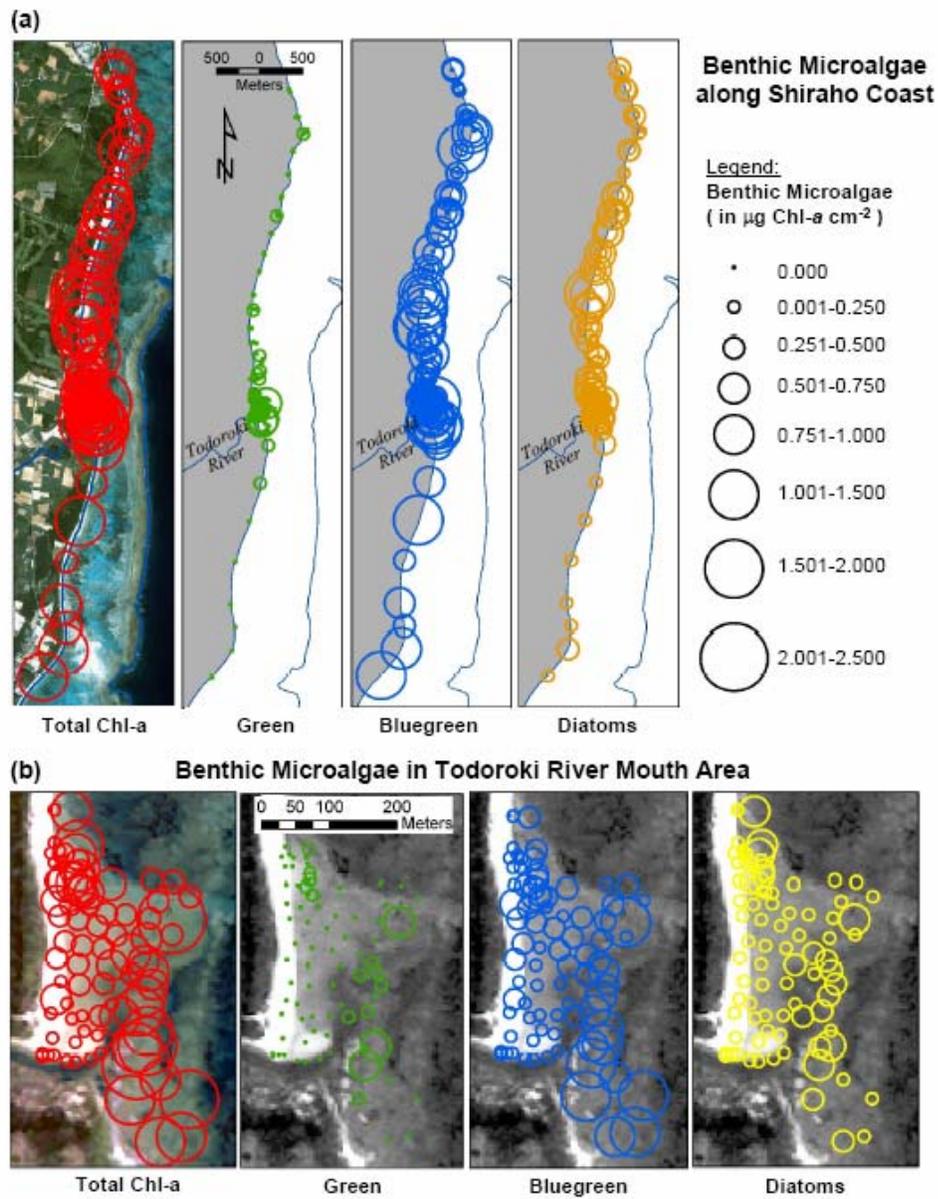


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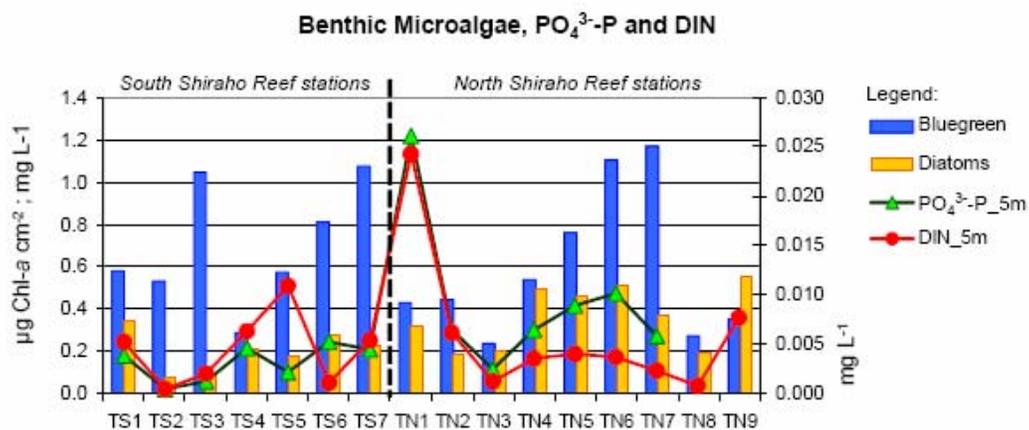


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