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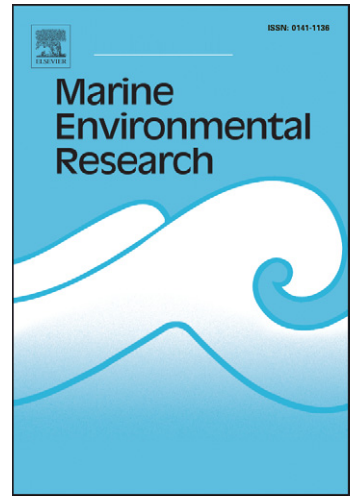

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PII:	S0141-1136(08)00198-0
DOI:	10.1016/j.marenvres.2008.08.005
Reference:	MERE 3280
To appear in:	*Marine Environmental Research*
Received Date:	9 March 2008
Revised Date:	31 July 2008
Accepted Date:	12 August 2008

Please cite this article as: Blanco, A.C., Nadaoka, K., Yamamoto, T., Planktonic and Benthic Microalgal Community Composition as Indicators of Terrestrial Influence on a Fringing Reef in Ishigaki Island, Southwest Japan, *Marine Environmental Research* (2008), doi: 10.1016/j.marenvres.2008.08.005

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

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Abstract Microalgal-based indicators were used to assess terrestrial influence on Shiraho coral reef of Ishigaki Island (Okinawa, Japan). A typhoon occurred on 4-5 August 2005 and sampling were made on three occasions thereafter (6, 8, 11 August). Pre-typhoon sampling was conducted on 26 July. The typhoon-enhanced terrestrial discharges increased reef nutrient levels (e.g. average NO_3^--N: 0.088 mg/L pre-typhoon to 0.817 mg/L post-typhoon). This elevated chlorophyll-*a* concentrations by four times and shifted phytoplankton composition (spectral class-based) from an initial dominance of diatoms and green microalgae to the dominance of bluegreen microalgae (cyanobacteria) and cryptophytes. Cyanobacteria later increased by more than 200% and accounted for as much as 80% of total chl-*a* (~0.29 μg/L), possibly assisted by favorable nutrient availability. In outer reef waters, diatoms and green microalgae predominated whereas cyanobacteria and cryptophytes were nearly undetectable. Due to detrital decomposition and river discharge, the CDOM was much higher in the inner reef than in the outer reef. Benthic bluegreen microalgae were relatively more abundant in areas close to the river mouth and coastal agricultural fields. At these locations, nutrient

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

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

1. Introduction

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

Eutrophication can lead to 'phase shift' or the transformation of a reef from being coral-dominated to algae-dominated. Early detection through monitoring can help prevent the occurrence of nuisance filamentous algae and other deleterious effects on corals. Integrated methodologies to assess coastal ecosystem condition and eutrophication status are warranted. A new set of ecologic indicators must be developed to fully measure coastal system complexities (Niemi et al., 2004). Proposed indicators include biological community approaches to detect effects on ecosystem structure and function. Microalgae are known to respond to changes in environmental conditions (e.g., nutrient availability, sedimentation, hydrology, irradiance, and temperature regimes) over a wide range of temporal scales (e.g., hours to even years). In addition, 'the ecological effects of environmental stressors are often evident at the microbial level, where the bulk of primary production and biogeochemical recycling occurs' (Paerl et al., 2003). However, information on the influence of environmental perturbations on changes at the base of the food web that mediate productivity and nutrient cycling are scarce (Paerl et al., 2003). Chlorophyll-*a* (chl-*a*) is the most commonly used indicator of eutrophication. However, since chl-*a* is a bulk indicator, it can only provide limited information and must be used with caution (Andersen, 2006). Various studies have suggested the use of microalgal functional groups or phytoplankton community composition as indicators of coastal ecosystem condition, since each group responds differently to nutrient availability and environmental conditions (Pickney et al., 2001; Paerl et al., 2003). Nutrient enrichment can result in changes in the phytoplankton community (Piehler et al., 2004), especially in areas subject to episodic nitrogen enrichment due to pulses of nitrogen-rich river discharge (Ornolfsdottir et al., 2004). However, little information is available on the phytoplankton community of coral reef waters (van Duyl et al., 2002; Tada et al., 2003). Differentiation of algal groups is also necessary to establish whether the reef environment influences phytoplankton composition (van Duyl et al., 2002).

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

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

Fig. 1

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

2. Materials and Methods

2.1 Study site

Shiraho Reef is a well-developed fringing reef with typical topographic features such as moat, reef pavement, reef crest, and reef edge (Kayanne et al., 1995). Average water depth in the moat is about 2 m. The reef crest emerges at low tides, during which the average water depth in the moat is about 1.5 m (Nadaoka et al., 2001). The width of the fringing reef varies from 0.7 to 1.4 km. Four channels exist in the reef, namely, 'Tooru-guchi', 'Ika-guchi', 'Moriyama-guchi', and 'Bu-guchi' (Fig. 1). Tooru-guchi is the largest channel, penetrating deeply into the reef and having an average depth of 20 meters (Tamura et al., 2007). Two rivers drain into the Shiraho Reef, the Todoroki and Tooru Rivers. The Todoroki River supplies large amounts of sediment and nutrients from the Todoroki watershed. The Tooru River drains towards the Tooru-guchi channel. Seagrass and macroalgae proliferate in nearshore areas north of the river mouth. Corals are scattered throughout the reef flat and are abundant in the reef slope (outer reef). The typical flow circulation pattern (tide-averaged velocity) in the reef (Fig. 22a in Tamura et al., 2007) indicates an evident convergence of mean currents toward Tooru-guchi and subsequent flow offshore. Flows in and near Moriyama-guchi and Ika-guchi are mostly directed offshore. Most importantly, the flow pattern near the Todoroki River mouth indicates that the current is directed northward. Water flows towards Moriyama-guchi and further northwards to Tooru-guchi. The pattern of currents in Shiraho Reef under small wave conditions is almost the same as that during high wave conditions (Tamura et al., 2007), thus, the flow pattern described can be considered representative. Particle tracking simulations showed that suspended particles from Todoroki River mouth are transported towards Tooru-guchi (Tamura et al., 2007). These characteristics can also have significant influence on nutrient transport within the reef.

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

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

2.2 Methodology

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

2.2.1 Reef water quality monitoring

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

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

(Yoshino Keisoku Co.,Ltd., Japan). Samples were stored in the dark in cooled containers while in the field. Samples for dissolved nutrient analysis were filtered through 0.45 μm acetate membrane filters (Advantec Dismic 25CS045AN). Filtrates were immediately frozen. Concentrations of dissolved nutrients (PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+, and SiO_2) were later determined using a Bran-Luebbe TRAACS 2000 auto-analyzer (Bran and Luebbe, Norderstedt, Germany). For chlorophyll-a analysis, samples were filtered using Whatman GF/F (0**.7**-μm pore size, 25mm diameter) glass microfiber filters . Chlorophyllous pigment was extracted using N,N dimethylformamide following the method of Suzuki and Ishimaru (1990). Chlorophyll-a content in the extract was then analyzed based on the method of Strickland and Parsons (1972) using a calibrated Turner fluorometer.

2.2.2 Measurement of planktonic microalgae

Class-differentiated algal measurements were conducted using a bbe FluoroProbe system (bbe Moldaenke, Kiel, Germany). This submersible instrument makes use of the fluorometric characteristics of microalgae to determine their class and concentration. FluoroProbe can differentiate phytoplankton into four spectral classes: ‘green’ algae (Chlorophyta), ‘blue’ algae (bluegreen algae or phycocyanin-containing cyanobacteria), ‘brown’ algae (Chromophyta, Dinophyta), and ‘mixed’ (Cryptophyta). The relative amount of colored dissolved organic matter (CDOM), also known as yellow substance, is measured to correct for the influence of the substance on microalgal fluorescence. Briefly, the instrument performs sequential light excitations using five light emitting diodes (LEDs) emitting at different wavelengths (i.e., 450, 525, 570, 590, and 610 nm) (Beutler et al., 2002). Subsequent measurements of relative fluorescence intensity of chl-*a* at 680 nm are then made immediately after each light excitation. By comparing the measured fluorescence excitation spectra or ‘fingerprints’ for the different algal classes with the calibration excitation spectra stored in the instrument, the relative amounts of each algal class are calculated using an algorithm. The manufacturer-provided calibration data were obtained by quantifying chl-*a* amounts in mixtures of laboratory cultures using high-performance liquid chromatography (HPLC). The mixtures were composed as follows: 'green' – *Scenedesmus* sp., *Chlamydomonas* sp., *Monoraphidium* sp., *Chlorella* sp., *Micratinium* sp.; 'blue' – *Mycrocystis* sp., *Synechococcus* sp., *Aphanizomenon* sp., *Anabaena* sp.; 'brown' – *Cyclotella* sp., *Nitzchia* sp., *Synedra* sp., *Ceratium* sp., *Peridinium* sp.; 'mixed' – *Cryptomonas* sp. FluoroProbe can measure algal concentrations up to 200 μg chl-*a* l^{-1} with a nominal resolution of 0.05 chl-*a* μg l^{-1}. FluoroProbe data were found highly correlated with cell counts and spectrophotometric total chl-*a* measurements (see Leboulanger et al., 2002; Gregor et al., 2005), indicating the reliability of the instrument. In this study, community composition refers to the spectral algal classes based on the FluoroProbe system rather than the traditional classification.

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

In summer 2006, phytoplankton concentrations were measured again with the objective of assessing differences in microalgae composition and concentration between inner reef and outer reef areas (20-40 meters deep). Profiles of algal concentrations in the water column were obtained at various points in the inner and outer reef areas before and after several rainfall events. The first measurements at the outer reef and inner reef were respectively conducted on August 16 and August 17, 2006, days after several minor rainfall events. On August 23, 2006, stronger rains occurred and several days after this event, the second set of measurements at the inner and outer reefs was conducted on August 29 and August 30, respectively.

2.2.3 Assessment of groundwater discharge zones

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

2.2.4 Measurement of benthic microalgae

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

2.2.5 Data Analysis

Field measurement and monitoring data were analyzed using graphical and statistical techniques. The data were organized and visualized in a geographic information system (ArcGIS^{TM} 9, ESRI Inc.) to see the spatial distribution of variables measured and the relationships between them across geographical space. Relationships between two variables were examined using bivariate correlation analysis with significance testing. Multiple factor analysis (MFA; see Escofier and Pages, 1994) was used to investigate in more detail the relative influence of nutrient supply and availability/limitation on phytoplankton concentration and class composition using data from summer 2005. MFA was utilized for simultaneous analysis of several tables (groups/sets) of variables. Using this method, common structures present in all or some of the sets of variables can be found. MFA makes use of successive application of principal components analysis (PCA). PCA is first applied to each data set. Each data set is then normalized. The normalized data sets are then merged to form a unique matrix on which a global PCA is applied (Abdi and Valentin, 2007). MFA was performed using XLSTAT (an MS Excel add-in). Three tables were used: phytoplankton concentration/composition, nutrient concentration, and nutrient (molar) ratios.

3. Results

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

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

Fig. 2

3.2 Spatial distribution of nutrients in the inner reef

In general, no appreciable differences in nutrient concentrations between near-surface and near-bottom samples in the inner reef were observed before and after the typhoon. Prior to the typhoon, low levels of nutrients were observed in general. However, relatively higher concentrations were found at stations R12 for almost all forms of dissolved nutrients (Fig. 3). Nitrate concentrations were relatively high at stations located near agricultural fields, particularly R5 and R7. After the typhoon, the spatial distribution significantly changed with PO_4^{3-}-P, SiO_2-Si and NO_3^--N concentrations generally increasing. The highest concentrations and rates of increase for these nutrients occurred at stations R5, R7, and R8, located immediately north of the Todoroki River's mouth. NO_3^--N and SiO_2-Si concentrations also increased significantly at R9 and R10. Increased nutrient concentrations were also seen in Tooru-guchi channel, specifically NO_3^--N, PO_4^{3-}-P and SiO_2-Si, due to Tooru River discharge. However, the increases were minimal compared to those observed at the stations close to the Todoroki River discharge area. At station R3, only minimal changes in nutrient concentrations were observed. Interestingly, NH_4^+-N concentrations did not exhibit considerable change before and after typhoon.

Fig. 3

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

FluoroProbe-measured total chl-*a* correlated well (r=0.87, $\rho<0.05$) with chl-*a* concentrations measured using laboratory methods. Before the typhoon, there was a clear distinction in the distribution of phytoplankton communities between the northern reef stations and the southern reef stations (Fig. 4a). The phytoplankton community composition at most stations north of the Todoroki River mouth (with the exception of R8) was characterized by the dominance of diatoms and green microalgae on July 26, 2005. In contrast, bluegreen microalgae dominated at stations south of the river mouth. Relatively higher contributions of cryptophyta were also observed at these stations. Increased nutrients in the reef due to river discharge after the typhoon resulted in an overall increase in total chl-*a* level (as much as 4-fold) and also a noticeable increase in bluegreen and cryptophyta concentrations, particularly in areas where diatoms and green algae previously dominated (north of the Todoroki River mouth) (Fig. 4b, 4c). At these areas, increased nutrient levels, notably nitrate and phosphate, were observed. Considerable increases in green microalgae and diatom contribution were observed in reef waters south of the Todoroki River mouth. The proportion of bluegreen microalgae increased further a few days later. On August 11, bluegreen microalgae accounted for as much as 80% of the total chl-*a* (Fig. 4d).

<u>Fig. 4</u>

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

On August 16-17, 2006, depth-averaged chl-*a* levels (FluoroProbe-measured) were notably higher in the outer reef compared to those in the inner reef (Fig. 4e) with exceptions at two stations – one near the Todoroki River mouth and the other near one of the drainage outlets. In the upper 2 meters of the water column, chl-*a* in the outer reef was as low as that in the inner reef (about 0.1 μg L^{-1}). Outer reef chl-*a* profiles (Fig. 5) indicated increasing concentration from less than 0.1 μg L^{-1} (near-surface) to about 0.3–0.4 μg L^{-1} (at maximum measurement depth of 15–20 meters). The vertical distribution of phytoplankton exhibited some degree of dependence on water density (σ_t), with higher phytoplankton biomass at deeper layers of higher σ_t. Phytoplankton community compositions in the inner reef and outer reef were notably different. Inner reef microalgal compositions varied greatly from station to station, with three to four microalgal groups present at each. At outer reef stations, diatoms (the most abundant) and green microalgae were dominant and the percentage contributions from bluegreen microalgae and cryptophytes were negligible. A major rainfall event occurred on August 23, considerably increasing river discharge. Total chl-*a* in the inner reef increased significantly at most stations based on the August 29 measurements. Depth-averaged chl-*a* concentrations in the inner reef became comparable or even higher than those in the outer reef. The phytoplankton community composition changed as well. Diatoms became dominant in the northern part of the inner reef. Green microalgae and diatoms dominated in areas near the Todoroki River mouth. On the other hand, the composition in the outer reef remained almost unchanged though the chl-*a* concentrations changed. The water column became more stratified, resulting in a more defined gradation of phytoplankton biomass, especially diatoms. For both measurement periods, diatoms and green microalgae dominated outside the reef with depth-averaged concentrations in the ranges of 0.037-0.233 μg L^{-1} and 0.019-0.146 μg L^{-1}, respectively, while bluegreen microalgae and cryptophytes were at near-zero levels.

Fig. 5

3.5 Diurnal variation of planktonic microalgae

Data from the one-day deployments of FluoroProbe at stations R1, R9, and R12 revealed dynamic diurnal variations in chl-*a* concentration and phytoplankton community composition in the inner reef (Fig. 6). Measurements at R12 were made before the typhoon while at R1 and R9; measurements were made after the typhoon. Since nutrients were not measured or monitored at these sample times, only the influence of other variables (e.g., turbidity, solar radiation) is presented. The overall level of total chl-a concentration was highest at R9, which was located to the immediate south of the Todoroki River mouth. At station R1, the total chl-a level was higher on August 11, 2005 compared to levels the following day, possibly due to higher solar radiation in the previous day. Chl-a levels peaked a few hours after the maximum solar radiation and gradually declined during the night. Diatoms started to dominate during low tide from around 15:30 on August 11. The wind velocity was relatively high throughout the R1 monitoring period at about 8 m s^{-1}. During the night, increased concentrations of cryptophytes and cyanobacteria were detected. Green microalgae were only present during the day. At station R9, the relatively high turbidity generally increased during the monitoring period due to the Todoroki River plume. Initially, cryptophytes and diatoms dominated starting from around 5 PM. Cryptophytes were predominant when light levels were low and turbidity was high. These were replaced by cyanobacteria, which were evidently dominant during the night and also during the following day when turbidity increased due to river plume. Cyanobacteria reached 0.8 μg L^{-1} after 17:00 on August 15. The concentration and percentage contribution of cryptophytes and diatoms were higher on August 14 than on August 15. Wind speed was relatively higher on August 14. Similarly, peak chl-a levels at station R12 lagged behind the peak solar radiation levels. Diatoms dominated at this station. Green microalgae were present only during the day. Cyanobacteria were again detected during the night with concentrations much lower than those observed at R1 and R12.

Fig. 6

3.6 Exploratory statistical analysis of phytoplankton and nutrient relations

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

For microalgal concentrations on July 26, 2005 (pre-typhoon), diatoms were closely associated with the nutrient ratios SiO_2/PO_4^{3-}, SiO_2/DIN, and NH_4^+/PO_4^{3-} (Fig. 7a). DIN is dissolved inorganic nitrogen (NO_3^--N, NO_2^--N, and NH_4^+-N). SiO_2 concentration per se seemed to be less important than SiO_2/PO_4^{3-} and SiO_2/DIN for diatoms. Bluegreen microalgae and cryptophyta were closely associated with each other. However, no clear association with specific nutrient levels and ratios could be inferred. On August 8, 2005, bluegreen and green microalgae had similar distributions, and were associated with most nutrients (i.e., NO_3^-, PO_4^{3-}, SiO_2, DIN) and to the nutrient ratios SiO_2/NH_4^+ and NO_3^-/PO_4^{3-}. Bluegreen algae exhibited negative relationships with SiO_2/DIN, SiO_2/NO_3^-, and NH_4^+/PO_4^{3-} on both occasions. Correlations of total chl-*a* and green microalgae with F1 were similar to that of NO_3^-/PO_4^{3-} on both occasions. The interplay among the variables resulted in the clustering of similar stations. On July 26, 2005, most stations, except R3 and R12, formed a cluster in the factor space (Fig. 7a). On August 8, 2005, R7 and R8, the stations to the immediate north of Todoroki River mouth, formed a separate cluster.

The percentage of bluegreen microalgae and cryptophyta seemed to have similar distribution for most stations on July 26, 2005 (Figs. 4a, 7b). The same can be said for the percentages of diatoms and green microalgae. However, %Bluegreens was inversely related (diametrically opposite) to %Diatoms just as %Cryptophyta was to %Green. In general, this implies that stations with relatively high percentages of bluegreen (/green) microalgae were likely to have relatively low percentages of diatoms (/cryptophyta). %Bluegreen and %Cryptophyta were associated mainly with PO_4^{3-} concentration. %Diatoms and %Green were related to NH_4^+/PO_4^{3-} primarily and also to SiO_2/DIN, SiO_2/NO_3^-, and SiO_2/PO_4^{3-}. On August 8, 2005, %Bluegreens showed a closer association with NO_3^-/PO_4^{3-}. Note again that %Diatoms and %Cryptophyta were diametrically opposite to %Bluegreen and to %Green, respectively. The clustering of stations on July 26 was similar to previous observations, with R3 and R12 being distinctly different from the other stations. On August 8, most stations formed a cluster with the exclusion of R5, R7, R8, and R10.

Fig. 7

3.7 Distribution of CDOM in reef waters

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

Fig. 8

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

3.8 Into-reef groundwater discharge assessment

Nutrient concentrations of samples taken at a distance of around 5 meters from the low water line were generally higher than those taken at about 20 meters (Fig. 9a). Among the northern stations (TN1-TN9), nitrate concentrations (5m and 20m) were relatively high at stations TN9 (relatively closer to the Todoroki River mouth) and TN5. Nitrite (5m) was highest at TN9 and generally decreased towards TN3. For stations on the southern coast (TS1-TS7), NO_3^- concentrations were highest at TS4 and TS5, and comparable to NO_3^- levels at TN9. NH_4^+ peaked at TS3 (5m) while NH_4^+ (20m) was relatively higher at TS4 and TS5. PO_4^{3-} concentrations (5m) were greater at TS3 and TS4 than at any other southern coast station.

The salinity was generally lower for water samples collected closer (i.e., at 5m) to the low water line than for samples collected farther away (i.e., at 20m) (Fig. 9b). Groundwater seepage was visible at several sampling and measurement locations along the beach. The lower salinity values at station TN5, TS5 and other nearby stations indicated that these were zones of relatively high groundwater discharge. The salinity correlated strongly and significantly ($\rho<0.05$) with NO_3^- concentrations with r=-0.87 and r=-0.88 for stations TN1-TN9 and TS1-TS7, respectively.

Fig. 9

3.9 Distribution of benthic microalgae

The river mouth area was dominated by bluegreen algae (most abundant) and diatoms (Fig. 10b). Similar high magnitudes of bluegreen algae were found on the coast adjacent to agricultural fields north of the river mouth and at the southernmost part of Shiraho reef, relatively close to residential areas (Fig. 10a). Benthic green microalgae were much lower in concentration than cyanobacteria or diatoms. They were absent or undetectable along some parts of the coast. Even in the vicinity of the Todoroki River mouth, where green macroalgae were abundant during the measurement period, the concentrations of green microalgae were less than 0.75 μg cm^{-2}. In contrast, the concentrations of cyanobacteria and diatoms were higher at parts of the river mouth where green macroalgae were observed.

Fig. 10

For the north coast stations (TN1-TN9), diatoms were positively related to PO_4^{3-}-P and also to NH_4^+-N (not shown) to some extent (Fig. 11). Bluegreen microalgae appeared to be influenced by PO_4^{3-}-P as well. The spatial variation for NO_3^--N very much resembled that of diatoms. For stations TS1-TS7, the distribution of diatoms corresponded strongly and significantly with PO_4^{3-}-P concentrations ($r = 0.81$, $\rho<0.05$). The spatial variations of diatoms and NH_4^+-N (not shown) for TS1-TS7 were also found similar. Considering all station, the overall trends of bluegreen algae, diatoms, DIN, and phosphate were similar to a certain extent.

Fig. 11

4. Discussion

4.1 Reef Nutrients and the Phytoplankton Response

The similarity of pre-typhoon and post-typhoon spatial distribution of NH_4^+-N concentrations indicated that NH_4^+-N is mainly produced within the reef. Indeed, NH_4^+-N is emitted from sediments of seagrass beds, especially in summer (Miyajima, 2001). For the other nutrients, the observed spatial distributions may be largely influenced by reef currents, as they are in agreement with the patterns of current described earlier [see Nadaoka et al. (2001) and

Tamura et al. (2007) for detailed description]. Nutrients at R7 and R8 were greatly increased by northward currents that transported nutrients discharged from the Todoroki River. As shown by the MFA results, these stations were significantly different from other stations (Fig. 7b). At channel stations R4 and R5, where water exchange between reef and open sea is relatively great, nutrient concentrations were very low, with minimal change observed between pre-typhoon and post-typhoon nutrient levels. The strong current converging on the Tooru-guchi channel limited the influence of nutrient discharge from the Tooru River on the reef surrounding Tooru-guchi. Due to this pattern of currents, most of the sediments and nutrients from the Tooru River were directly flushed to the outer sea via the channel. This pattern was also responsible for the nutrient concentration at station R3, which remained low even after the typhoon. These observations indicate that much of the nutrients (except NH_4^+-N) in the reef came from the Torodoki River and were eventually transported and dispersed to the north by currents, especially during typhoons and strong rainfall events. Thus, advection and diffusion of river plume in the reef, and hence nutrients, are strongly governed by the relative locations of river mouths and channels, especially large channels like Tooru-guchi.

The increased nutrient levels in the reef elicited varied responses by microalgal communities at different locations. This is indicative of the complex relationship between nutrient availability and phytoplankton responses and also reflects the influence of hydrodynamics. The observed difference in phytoplankton composition (and the resulting change in class contribution) between reef waters north and south of the Todoroki River mouth can be attributed to the influence of reef hydrodynamics and nutrient source. The reef area north of the Todoroki River mouth is subjected to relatively greater influence by terrestrial runoff via discharge from the Todoroki River and also from agricultural drainage outlets near the coast. On the other hand, the southern reef area can be considered more influenced by groundwater discharge from the adjacent lands. The early dominance of green microalgae, cryptophytes, and diatoms (to a certain extent) in the nutrient-enriched reef waters after the typhoon was possibly due to the relatively efficient growth rates and enhanced uptake rates of these microalgal groups (Paerl et al. 2003; Delesalle et al. 1993). Green microalgae and cryptophytes are common in freshwater and brackish water rather than seawater. Consequently, the dominance of these microalgal classes at some reef stations after the typhoon may be thought as a result of enhanced freshwater input rather than higher nutrient availability. However, FluoroProbe measurements in the outer reef pointed to the presence of marine green microalgae. In the inner reef, cryptophytes were detected (and contributed significantly) even during low river discharge periods (Fig. 4a, 4e, 4f). FluoroProbe measurements made in Todoroki River indicated that cryptophytes is not a dominant algae class in the river. In addition, cryptophyte contribution to total chl-a in nearshore marine waters elsewhere was found to increase as well at low nutrient concentrations and high salinities (Tamigneaux et al., 1995). Further investigation through microscopic examination and phytoplankton pigment analysis by high-performance liquid chromatography (HPLC) is suggested.

The observed shift to cyanobacteria dominance may have been aided by grazing pressure (by zooplankton) as green microalgae are often more palatable than cyanobacteria. Silicon (Si) limitation may have also occurred, resulting in a decrease in diatom concentration and contributing to increases in the concentration and contribution of bluegreen algae (Rocha et al., 2002). This is possible as the increase in Si and P due to typhoon would have induced the growth of diatoms. As diatoms multiply, however, Si is consumed and may eventually become limiting relative to other nutrients once river flow reverts back to the normal flow level and groundwater discharge starts to diminish. At this stage, the availability of nutrients may favor the growth of cyanobacteria. This is supported by the MFA results, suggesting an inverse relationship of cyanobacteria percentage with SiO_2/DIN and SiO_2/NO_3^- before and after typhoon. On the other hand, diatoms have a relatively high specific gravity compared to other microalgae, making them better adapted to highly turbulent conditions than calm-water conditions. The cyanobacteria dominance after the storm may have been a consequence of decreased turbulent flow that should have led to sedimentation of diatoms, rather than depletion of Si. However, diatoms were observed to dominate the northern Shiraho reef on July 26, 2005, several days before the typhoon. MFA results indicated that diatom percentage was influenced by NH_4^+/PO_4^{3-}. The increase in PO_4^{3-} concentrations after the typhoon drastically reduced NH_4^+/PO_4^{3-} for all northern reef stations. This potentially led to the significant reduction in diatom percentage at stations R3, R4, R5, R6 and R7. Furthermore, a few days after the typhoon, diatom dominance was observed only at some reef stations, particularly those that were located near channels, where turbulence was usually high. It is important to note that the cryptophyta concentration and percent contribution generally increased immediately after the typhoon when turbidity was still high compared to calm-water conditions. This can be explained by the cryptophyte's motility and its ability to thrive in low light conditions (Bergmann, 2004). However, as light penetration of the water column increases, cyanobacteria may replace cryptophytes. Thus, proliferation of cyanobacteria several days after the typhoon was more likely caused by increased nutrient availability favorable to cyanobacteria and aided by improved light conditions.

As MFA results indicated, the percentage contribution to total chl-*a* by cyanobacteria is somehow inversely related to that of diatoms, potentially providing insights into the general phytoplankton succession in the reef. This was tested using the one-day continuous data obtained at R1, R9, and R12. Analysis of daytime (i.e., 7AM-5PM) data showed that the correlation between %cyanobacteria and %diatoms was significantly higher than other pairs with values of -0.56 (R1, Aug 11, 2005), -0.48 (R9, Aug 15, 2005) and -0.66 (R12, Aug 2, 2005). The correlations are not that strong as phytoplankton community composition is highly dynamic and subject to several complex interacting factors. Nonetheless, an inverse relationship exists between these two algal classes in Shiraho Reef, which requires further investigation.

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

4.2 Phytoplankton: Inner Reef vis-à-vis Outer Reef

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

Only a few studies have investigated the differences in phytoplankton community composition between coral reefs and the adjacent open sea. In Shiraho Reef, the four 'spectral' classes of microalgae were present. The near absence of cryptophytes in the outer reef can be due to extremely low organic matter (Fig. 11) in this area as cryptophytes are principally prevalent in areas with high concentrations of organic matter (Bergmann, 2004). Cryptophytes are able to maximize light absorption/utilization and capitalize on alternative fuel sources (e.g., organic nutrients) under low light conditions (Bergmann, 2004), hence the increased concentration of cryptophytes at low light level and increasing CDOM (Fig. 7). Cyanobacteria were found in the inner reef only due to the higher nutrient availability as described earlier. The difference in phytoplankton community compositions in the inner reef and outer reef may be partly explained by expelled zooxanthellae that occur only in the inner reef. The foregoing indicates that reef environments significantly influence phytoplankton composition primarily through nutrient availability and the abundance of organic matter.

4.3 Spatial and Temporal Distribution of CDOM in Shiraho Reef

CDOM protects reef organisms by absorbing harmful ultraviolet radiation, which is linked to coral bleaching (Otis et al. 2004). It is important that the spatial and temporal distribution of CDOM in the reef as influenced by terrestrial discharge and other factors be assessed. The observed increase in inner reef CDOM level after increased discharge from the Todoroki River indicates that river water is one of the primary sources of CDOM in Shiraho Reef. However, the observed difference between CDOM in river water and in inner reef water is not large (i.e., only 4-fold). On the other hand, the inner reef CDOM concentration is several times higher than that in the outer sea (Fig. 11). If CDOM in the inner reef were totally supplied by the river, the salinity of the inner reef water should be much less than that in the outer sea. However, this is not the case and CDOM must have come from sources within Shiraho Reef such as seagrass meadows, macroalgal colonies, and bottom sediments. CDOM can be derived from benthic habitats such as seagrass meadows (Stabenau et al., 2004). It can also be produced by heterotrophic bacteria through the alteration of algal-derived dissolved organic matter (Rochelle-Newall and Fisher, 2002). CDOM is produced by the decomposition of algal and seagrass detritus. The strong positive correlation between CDOM concentration and cyanobacterial concentration during the night-dawn transition may indicate that CDOM in the reef is derived from DOM precursors produced by cyanobacteria.

4.4 Groundwater Discharge, Nutrients, and Benthic Microalgae

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

The impact of nutrients derived from groundwater discharge on the reef is reflected in the spatial distribution of benthic microalgae along the Shiraho coast. In general, significant correlations and trend similarities were found between nutrient concentrations and benthic microalgae. Apart from the river mouth area, concentrations of both quantities were high in groundwater discharge zones previously identified. As there was no rainfall and subsequent discharge from the agricultural drainage outlets during the benthic microalgal measurement period, the high nutrient concentrations (concurrent with low salinity) measured during low tide can be attributed primarily to groundwater discharge. Benthic diatoms correlated well with the NO_3^- concentrations of water samples taken during low tides. However, the groundwater in the study area is also known to contain high concentrations of SiO_2-Si, ranging from 100-200 μM (Kawahata et al., 2000; Miyajima et al., 2007b). This, in addition to NO_3^-, may have benefited benthic diatoms inhabiting groundwater seepage areas. Nutrients may come from benthic habitats as well (e.g., NH_4^+ from seagrass bed sediments), thus increasing nutrient concentrations independently of groundwater. Nonetheless, benthic microalgae can be used for assessing the influence of groundwater seepage on the nearshore reef areas.

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

4.5 Utility of Microalgae for Assessing Terrestrial Influence on Coral Reef

In this study, the terrestrial influence on Shiraho Reef was explored and assessed in two modes – influence through terrestrial runoff and influence through groundwater discharge. Terrestrial influence was assessed through sampling of phytoplankton and benthic microalgae in addition to nutrients and sediments. We demonstrated that nutrient level and availability not only control phytoplankton concentration but also influence the community composition. The concentration and composition of phytoplankton in turn provides additional information on the factors affecting them, such as hydrodynamics and water quality (e.g., turbidity). The benthic microalgae concentration also corresponded to nutrient levels associated with groundwater discharge, particularly at the nearshore reef areas. Thus, the utility of microalgae as indicators of terrestrial influence on coral reef was convincingly demonstrated. In addition, the approach used in this study has a unique merit in that indicators can be measured in situ and in real-time. On declining reefs, cyanobacteria are increasingly prominent as they are adaptive to high nutrient concentrations, strong solar radiation, and higher temperatures (Hallock, 2005). Monitoring of phytoplankton community composition is thus important.

Conclusions

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

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

Acknowledgements The authors would like to express deep thanks to Takuma Nishimoto, Naoto Takemoto, Kazuma Arisaka, and Shinpei Matsuura for their assistance during field surveys. The authors are grateful to Prof. Jota Kanda and Prof. Takashi Ishimaru of Tokyo University of Marine Science and Technology for their support in water sample analysis. This research was supported by JSPS (The Japan Society for the Promotion of Science), Grant-in-Aid for Scientific Research (A) (No. 17206052 and No. 18254003), JSPS Core University Exchange Program and APN (Asia-Pacific Network) Grant (ARCP2006-08NMY-Nadaoka).

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Figure captions

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

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).

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.

Fig. 5. Typical class-differentiated phytoplankton profiles in outer Shiraho Reef in Summer 2006. Chl-a concentration is expressed in μg L-1. Plots correspond to the labeled stations in Fig. 4. CDOM in relative amounts (unitless) is also shown. Average water depth (H) at each station is indicated.

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

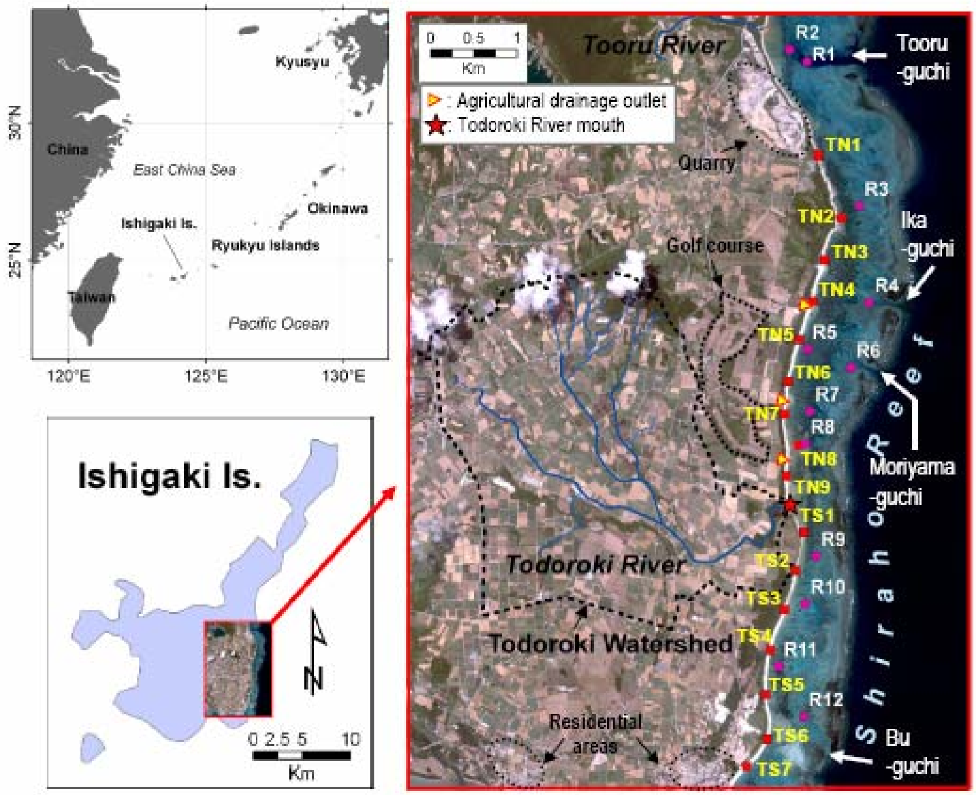
Fig. 7. Relationship between phytoplankton and nutrients (concentrations & ratios) in Shiraho Reef (inner) on 26 July (before typhoon) and on 8 August 2005 (after typhoon): (a) phytoplankton concentration and nutrient; (b) phytoplankton composition and nutrients. The percentage variability of the data 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.

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

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



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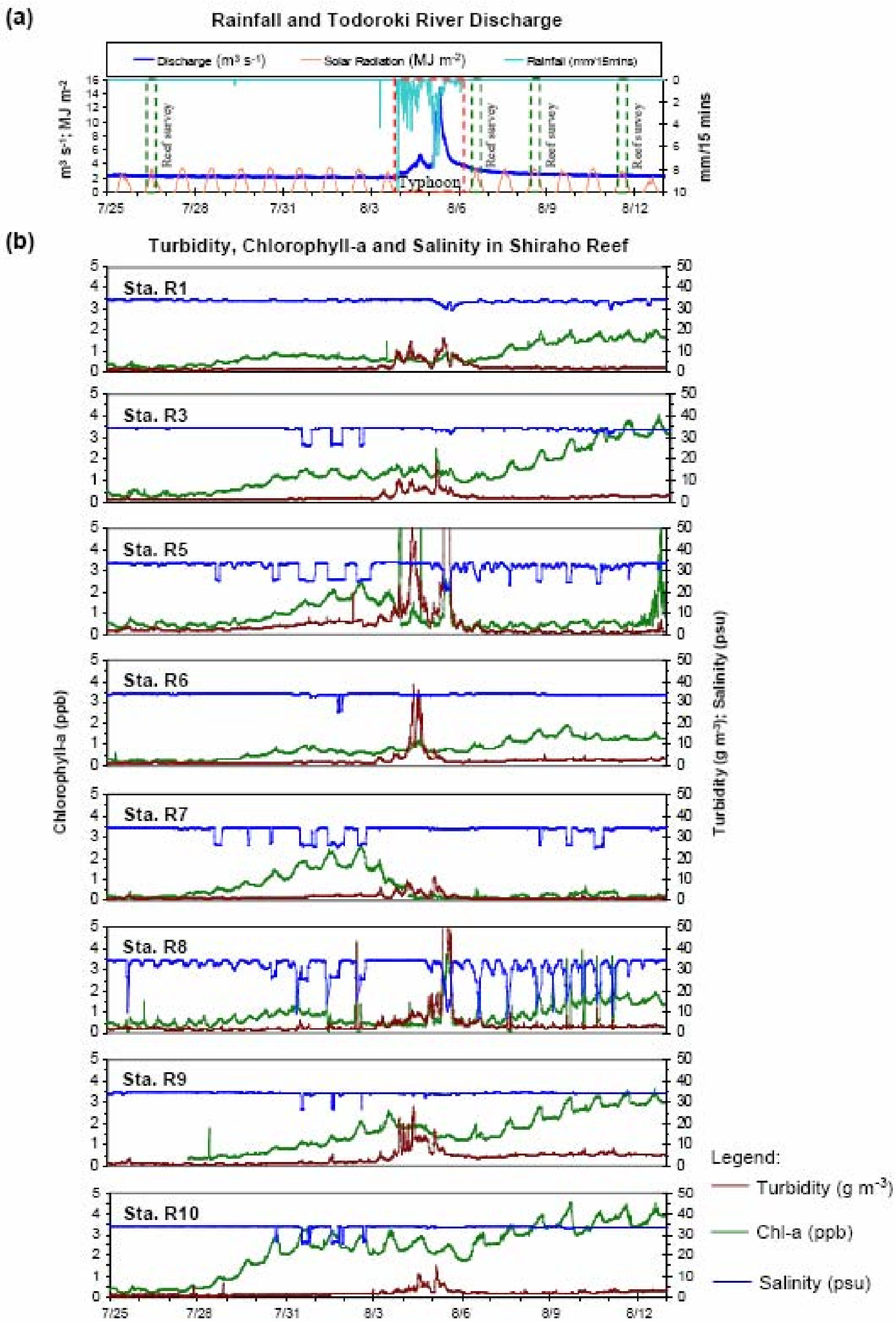

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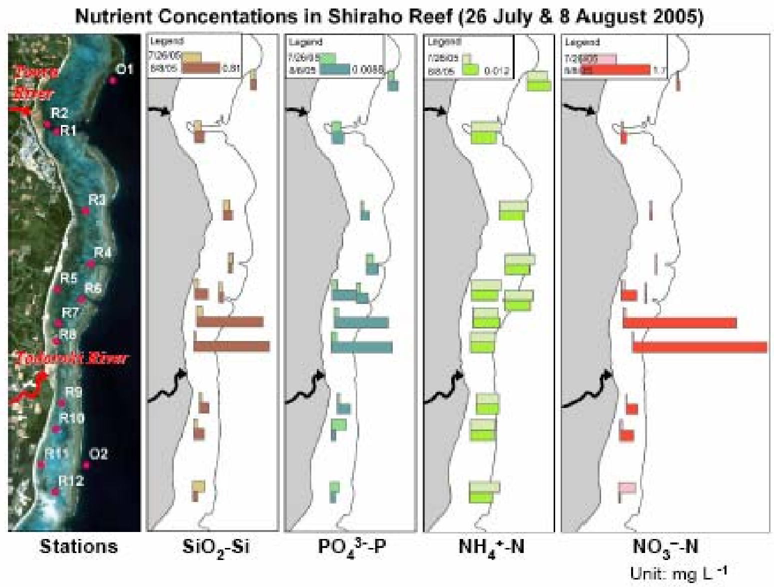

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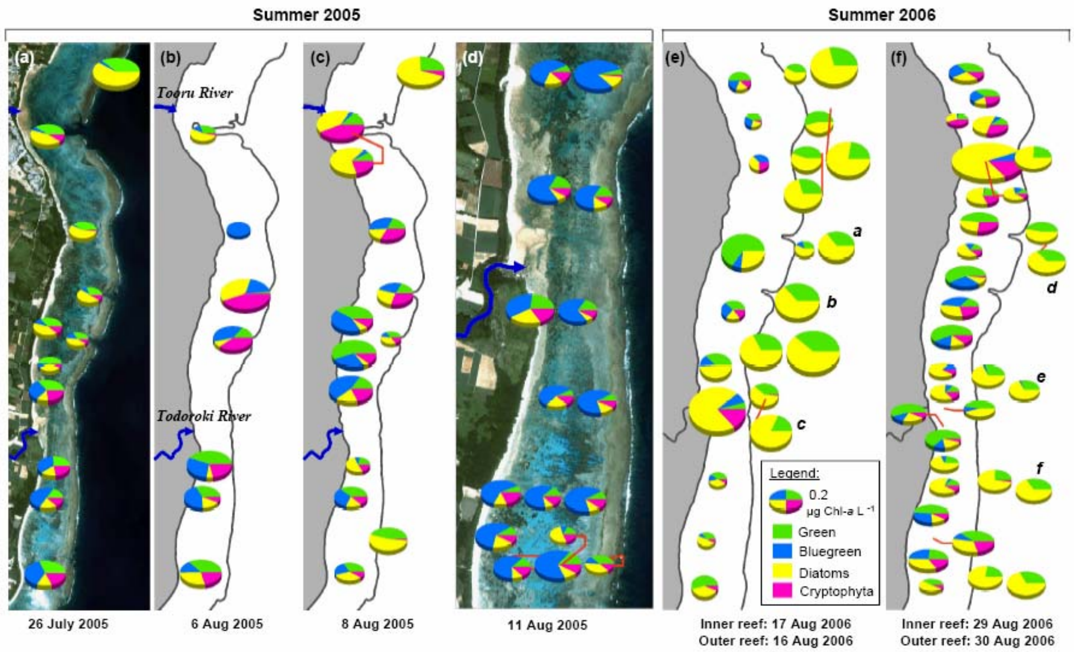

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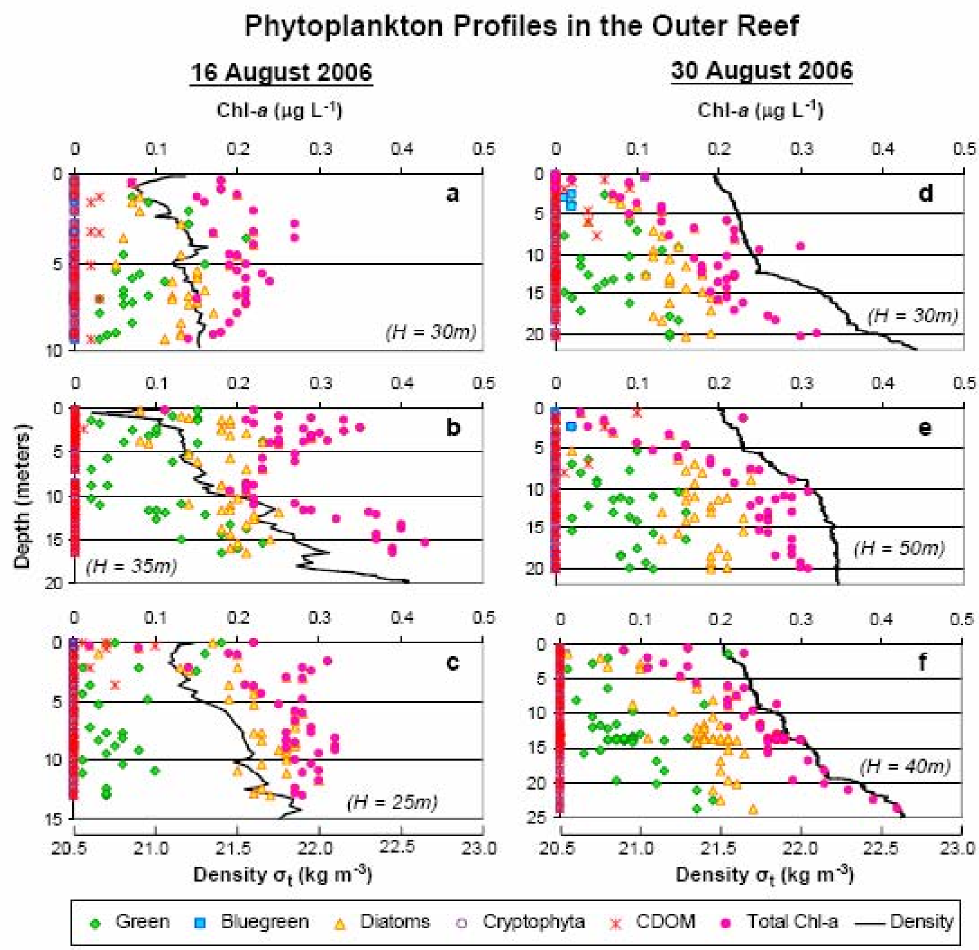

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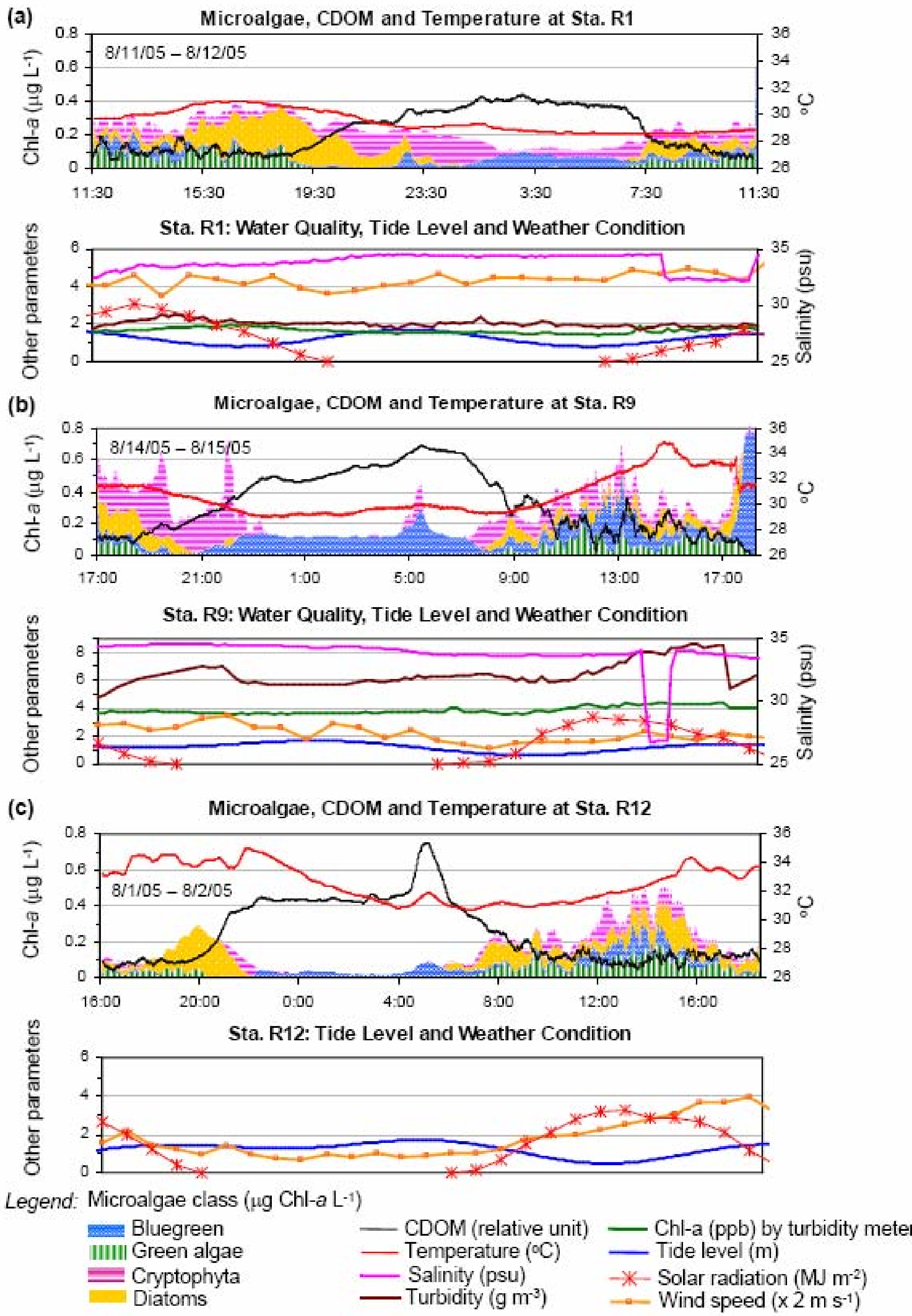

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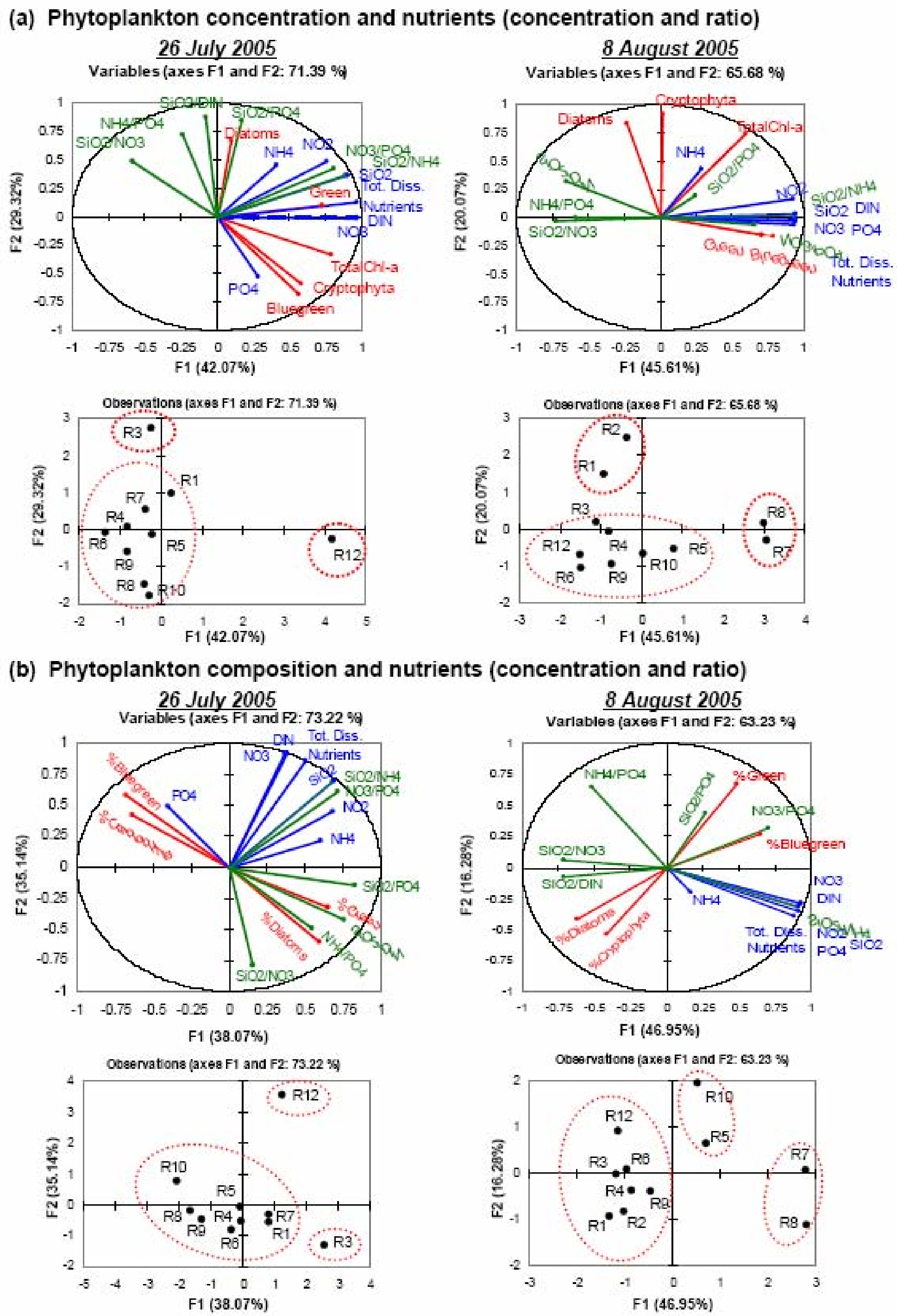

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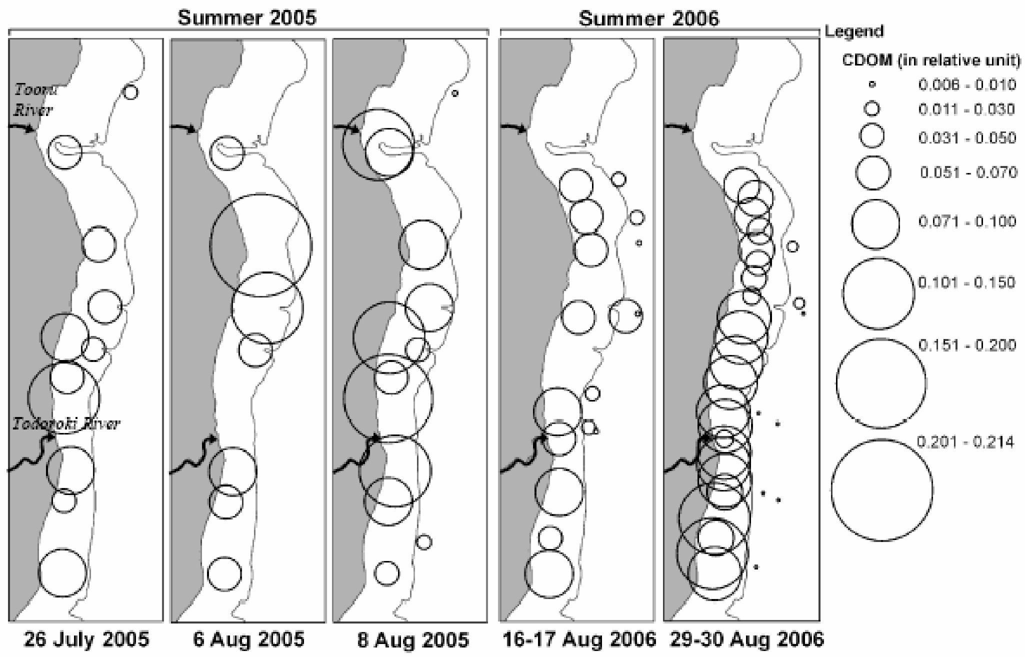

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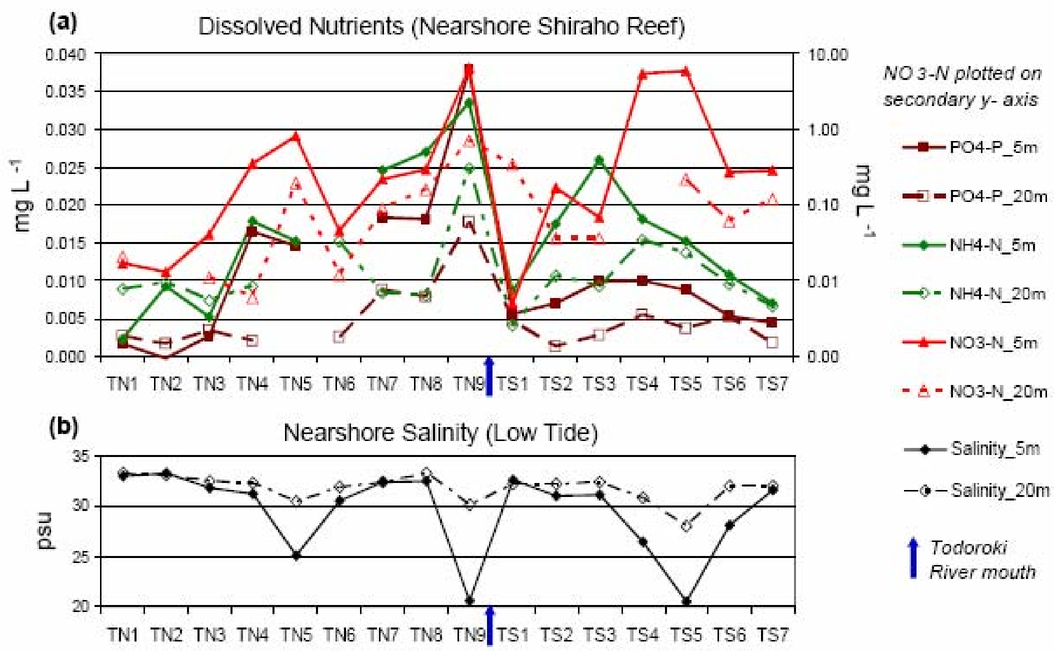

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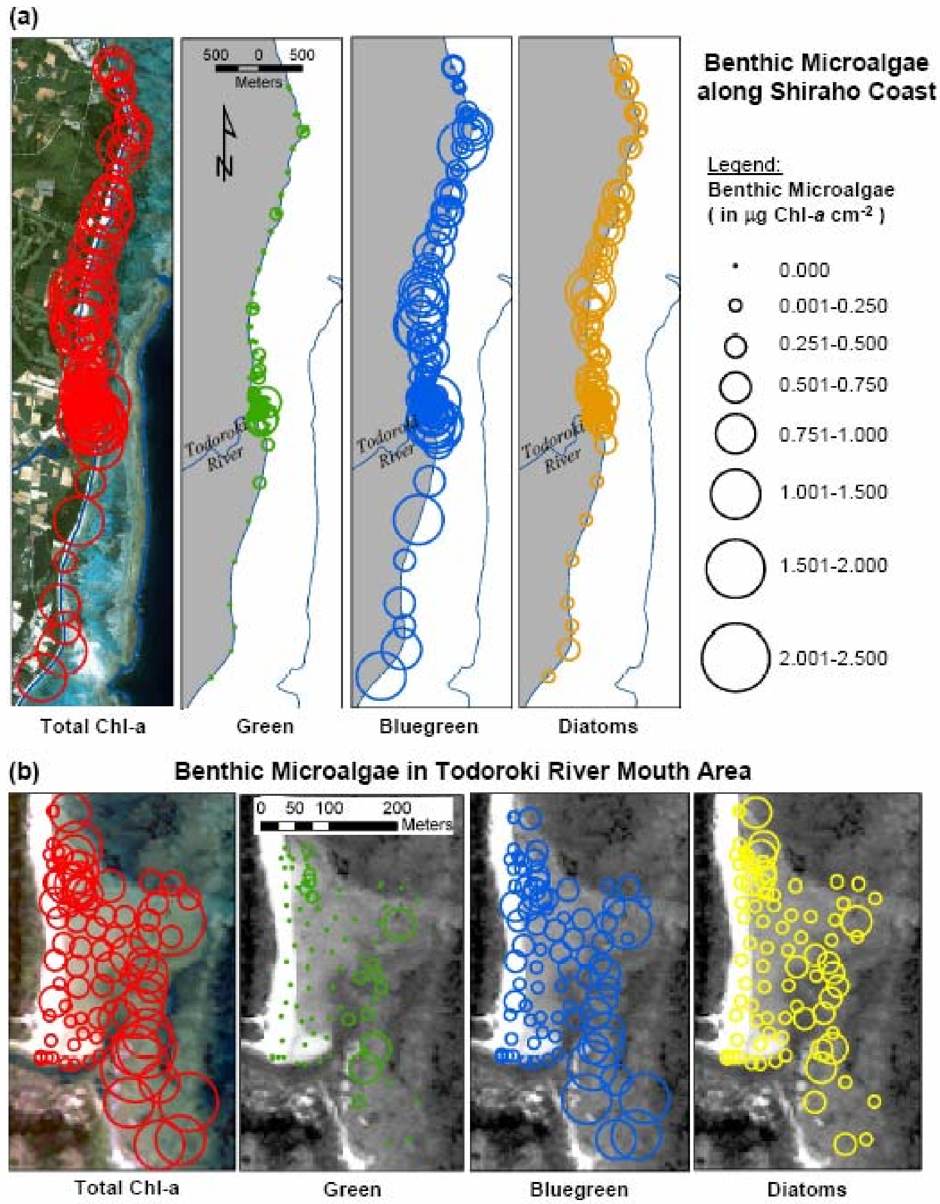

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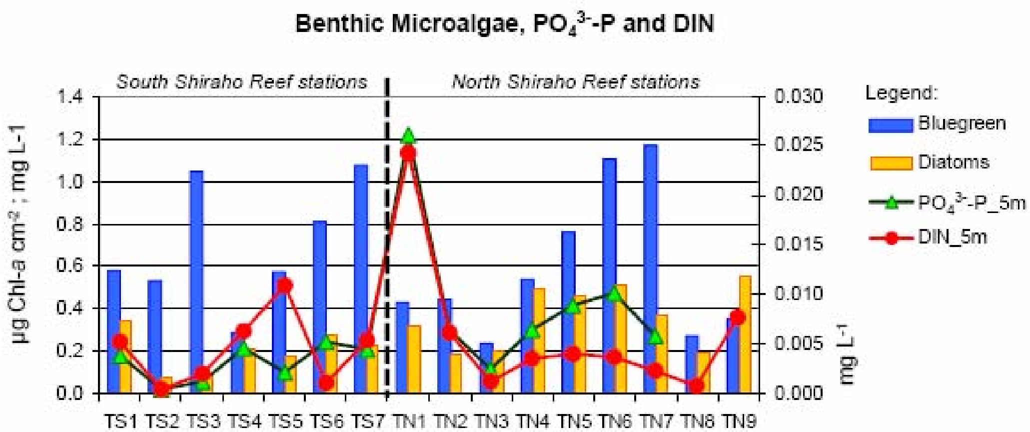

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