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Bacterial influence on chromophoric dissolved organic matter in coastal waters of the northern South China Sea

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ABSTRACT: Chromophoric dissolved organic matter (CDOM), bacterial production (BP) and phytoplankton biomass (chl *a*) were investigated at 2 coastal sites in the northern South China Sea during summer 2011. Our data showed that chl *a* levels were higher in Hong Kong eastern waters (HKEW) than Sanya Bay (SYB), but CDOM exhibited the opposite pattern. This decoupling between surface CDOM and chl *a* indicates that phytoplankton biomass is not the determining factor affecting CDOM distribution. The low levels of CDOM in HKEW could be associated with upwelling of CDOM-deficient deep waters. In addition, bacteria produced more CDOM during the beginning of incubation experiments at SYB than at HKEW, although CDOM turnover time by bacteria was ~4 to 10 d and showed no significant difference between SYB and HKEW ($p > 0.05$). Addition of nitrogen shortened the CDOM turnover time by 2 fold at SYB. Upwelling likely resulted in a phosphorus deficiency for bacteria, and phosphorus enrichment increased bacterial abundance (BA) and BP at HKEW. Interestingly, nitrogen enrichment increased both BP and CDOM generation in SYB, but phosphorus enrichment did not change CDOM at either location, suggesting that CDOM distribution is more associated with nitrogen than phosphorus availability.

KEY WORDS: Bacterial production · BP · Bacterial abundance · BA · Bacterial respiration · BR · Chromophoric dissolved organic matter · CDOM · Upwelling

INTRODUCTION

The fraction of dissolved organic matter that absorbs ultraviolet (UV) and visible light is referred to as chromophoric (or colored) dissolved organic matter (CDOM). CDOM is an important optical constituent in seawater, as it attenuates photosynthetic radiation and shields biota from harmful UV radiation. Hence, CDOM is crucial for organisms that are capable of photosynthesis (Sousa et al. 2008), and photochemical transformation of CDOM plays an important role in the cycling of carbon and other elements (Yin 2003). Understanding the dynamics of CDOM is also necessary in order to accurately inter-

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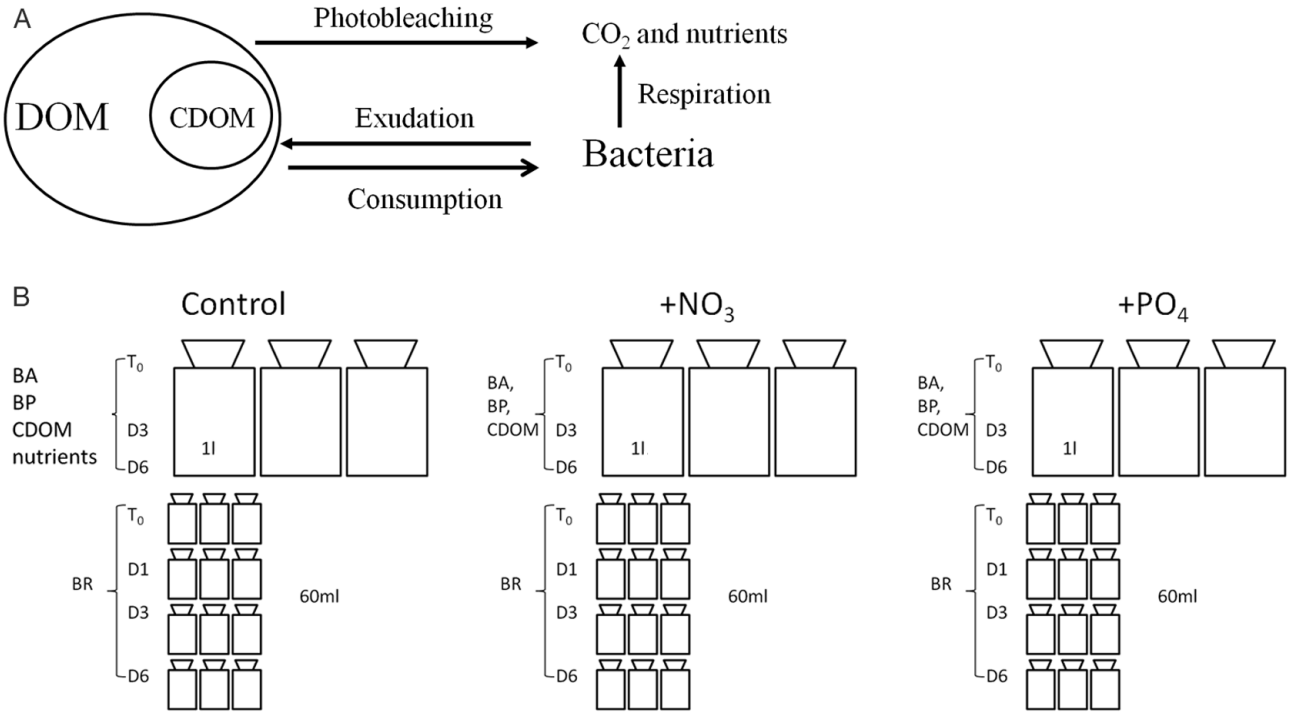


Fig. 1. (A) Conceptual model of the interactions between chromophoric dissolved organic matter (CDOM) and bacteria. (B) Experiment setup for the analysis of bacterial abundance (BA), production (BP), CDOM, and nutrients (1 l polycarbonate bottles, in triplicate) with and without the addition of nitrogen or phosphorus. For the analysis of bacterial respiration (BR), samples were incubated in the dark in 60 ml biological oxygen demand bottles with and without the addition of nitrogen or phosphorus, respectively, for 1, 3 and 6 d (D1, D3 and D6)

pret ocean color remote sensing data (Chen et al. 2003).

CDOM is produced as a by-product of the microbial breakdown of dissolved organic carbon (DOC) (Nelson et al. 1998). Bacteria can regulate dissolved organic matter (DOM) composition through the selective consumption of labile compounds and subsequent release of humic-like (chromophoric) substances, which emit radiation in the wavelength range of 380 to 420 nm when excited at 320 nm (Romera-Castillo et al. 2010). However, bacterial respiration (BR) and growth is usually associated with nutrient availability (Del Giorgio et al. 2011, Sinsabaugh et al. 2013), which consequently influences the microbial release and consumption of CDOM (Fig. 1A). Although Biers et al. (2007) reported that the distribution of CDOM is affected by nitrogen (N) availability, there is still little information about how the availability of other nutrients such as phosphorus (P) contribute to variation in CDOM.

Previous studies have reported that physical processes such as typhoons, cold eddies and upwellings can significantly alter the distributions of CDOM and CDOM fluorescence (fDOM) in offshore waters in the northern South China Sea (SCS) (Shang et al. 2008, Ma et al. 2011). Located in the northern SCS, Hong Kong eastern waters (HKEW) and Sanya Bay (SYB) waters are subject to terrestrial inputs of organic matter and nutrients from the Pearl River estuary (PRE) and Sanya River, respectively (Huang et al. 2003, Harrison et al. 2008, Yuan et al. 2010). The Pearl River is the second largest river in China, and its discharge reaches its annual maximum volume when NO_3^- concentration (~100 µM) and molar N:P ratios (~100:1) are high (Yuan et al. 2010). In addition to terrestrial inputs of nutrients, upwelled nutrients could provide a source of new nutrients in the upwelling system (Borges & Frankignoulle 2002). It has been reported that bacterial production (BP) and CO_2 levels in Hong Kong waters are also influenced by upwelling in the summer wet season, when surface waters flow offshore due to the southwest monsoon-induced winds (Yuan et al. 2011a). In contrast to HKEW, SYB is less influenced by upwelling in summer (Fig. 2A), which provides

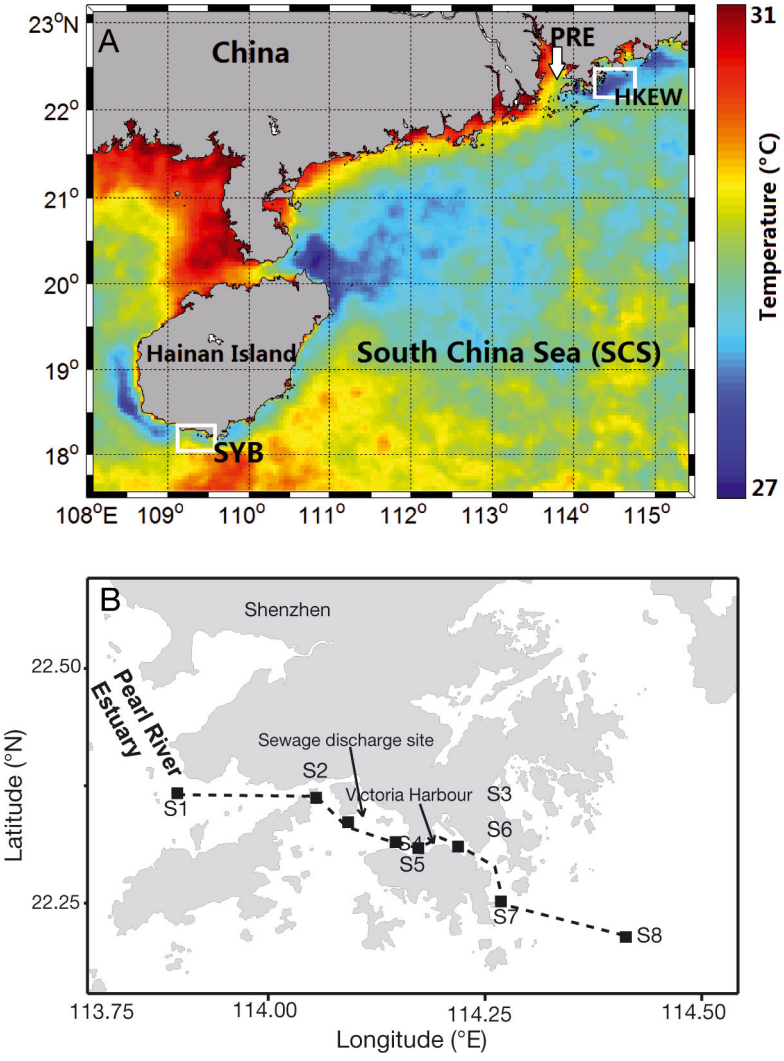


Fig. 2. (A) Sea surface temperature distribution derived from satellite data for the northern South China Sea during summer 2011 at experimental sites Hong Kong eastern waters (HKEW) and Sanya Bay (SYB) (white boxes). PRE = Pearl River Estuary (arrow). (B) Sampling stations along a transect from Stn 1 in estuarine waters to Stn 8 in HKEW

an opportunity to study the different effects of physical process on CDOM distribution in these 2 coastal water bodies.

In this study, we analyzed satellite data to examine the relationship between phytoplankton biomass and CDOM in summer 2011, in order to understand how CDOM is affected by upwelling and bacterial growth in dynamic coastal waters that are subjected to complicated hydrodynamics and significant anthropogenic forcing. To our knowledge, this is the first study of the effects of bacterial growth and nutrients on CDOM distribution in upwelled waters in the SCS.

MATERIALS AND METHODS

Satellite data

We derived seasonally (July to September) averaged sea surface temperature (SST), chlorophyll *a* (chl *a*) and CDOM data for the summer of 2011 from Standard Mapped Image (SMI) data (4 km spatial resolution) in the Distributed Active Archive Center (DAAC) of Goddard Space Flight Center (GSFC), NASA (http://oceancolor.gsfc.nasa.gov). Image analysis was carried out using the Matlab Image Processing Toolbox (V2012a). Many studies have reported that upwelling areas can be identified based on SST images (e.g. Sousa et al. 2008); hence, we present the location of upwelling areas with SST distribution (Fig. 2A).

Experimental setup

Our experiments were conducted at HKEW and SYB during the summer of 2011. Vertical profiles of salinity, temperature, and chl *a* were measured with YSI®6600 sensors; surface seawater was collected in carboys from 0.5 m below the surface in Port Shelter at HKEW and the southern waters at SYB (Fig. 2B). Once in the laboratory, the water from the carboys was filtered through precombusted Whatman GF/F filters (nominal pore size 0.7 µm) to remove phytoplankton and most heterotrophs. Bacterial regrowth cultures were carried out with and without the addition of N (NO_3) and P (PO_4) according to previous procedures (Yuan et al. 2011a); control culture received no nutrient additions. NO_3 and PO_4 were added to water samples in 1 l polycarbonate bottles for the analysis of bacterial abundance (BA), BP, nutrients and CDOM (Fig. 1B). The final concentrations in the NO_3 and PO_4 enriched treatments were about 50 and 5 µM, respectively. Subsamples for BA, BP, nutrients and CDOM were taken on Day 0, 3, and 6. In addition, 12 × 3 BR samples in biological oxygen demand (BOD) bottles were filled from the carboy with and without the addition of N or P, respectively (Fig. 1B). Initial samples (T_0) for BR measurement were immediately fixed and other samples were incubated for 1, 3, and 6 d (D1, D3 and D6). Samples were incubated in dark tanks with flowing surface seawater at 25.6 ± 2°C.

BA, BP and BR

Samples for BA (1.8 ml) were fixed using glutaraldehyde (2.5% final conc.) and stored in liquid nitrogen at −80°C. The samples were thawed immediately before analysis, diluted 10- to 100-fold in TE buffer (pH 8), and stained with SYBR green I (Molecular Probes) at 80°C in the dark for 10 min. Fluorescent microspheres (Molecular Probes) with a diameter of 1 µm were added to all samples as an internal standard. BA was quantified using a FACSCalibur flow cytometer (BD Biosciences).

BP (1.8 ml) was determined as described by (Simon & Azam 1989). ^{3}H-leucine (final conc. 30 nM; specific activity 55.9 Ci mmol^{-1}) was added to 2 ml subsamples (in triplicate) in microcentrifuge tubes (Axygen) with 1 control fixed by 5% cold trichloroacetic acid (TCA). The subsamples were incubated to determine leucine incorporation for 1 h and the linearity of the incorporation of leucine was tested in a separate time series experiment (data not shown). The incubation was terminated by adding TCA (5% final conc.). After centrifugation and aspiration of the supernatant, pellets were rinsed and centrifuged twice with 1 ml of 5% TCA, and the scintillation cocktail was added to the vial. The incorporated ^{3}H was determined using a Wallac 1414 liquid scintillation counter (Perkin-Elmer). The conversion factors (CFs) were determined to be 2 to 4 kg C mol leucine^{-1} according to Simon & Azam (1989).

Dissolved oxygen (DO) was analyzed using the high-precision Winkler titration method. Titrations were carried out in the laboratory with an automated titration apparatus (716 DMS Titrino, Metrohm®) with a potentiometric detector to determine the endpoint, following JGOFS protocols (Knap et al. 1996). The 60 ml custom-made BOD bottles were filled with seawater by overflowing ~4 or 5 times. Bacterial growth efficiency (BGE) was calculated from the ratio of BP/(BP + BR).

Chl *a*, inorganic nutrients and DOC

Chl *a* samples (200 ml) were measured before incubation using *in vitro* fluorescence with acetone extraction, and measured on a Turner Designs TD 700 fluorometer (Knap et al. 1996). Samples for nutrient analysis were taken with a 60 ml syringe and filtered through a pre-combusted Whatman GF/F filter mounted in a Swinnex filter holder and dispersed into Nalgene bottles (Thermo Fisher Scientific). All plasticware was pre-cleaned with 10% HCl. The filtered water samples were placed in a cooler with dry ice and frozen until analysis. Nutrients (nitrate, phosphate and silicate) were measured with a Skalar SAN autoanalyzer following Joint Global Ocean Flux Study (JGOFS) protocols (Knap et al. 1996). Molar ratios of dissolved inorganic nitrogen:dissolved inorganic phosphorus (DIN:DIP) were actually NO_3:PO_4 ratios since NH_4 was <1 µM, below our measurement limit.

Before incubation, DOC samples (100 ml) were placed in a cooler with dry ice and frozen until analysis. DOC concentrations were determined after water samples were filtered through 0.7 µm GF/F combusted filters and acidified with ~50 µl of 50% H_3PO_4 to pH <2 in order to drive off the inorganic carbon. The acidified samples were purged with ultra-high purity nitrogen immediately prior to analysis for approximately 10 min to remove any remaining inorganic carbon. Milli-Q water was used as the carbon-free distilled water blank. DOC concentrations were measured by high-temperature combustion using a Shimadzu TOC analyzer, which was filled with a Pt-coated Al_2O_3 catalyst at 680°C. Acidified samples were run for DOC and calibrated using the calibration standard, potassium hydrogen phthalate (KHP). The concentration of DOC was determined by subtracting the system blank area from the average peak area and dividing by the slope of the standard curve (Knap et al. 1996).

CDOM light absorption coefficients

CDOM samples were filtered through pre-combusted Whatman GF/F filters, and light absorption was measured using a Perkin-Elmer Lambda spectrophotometer and 1 cm quartz cuvettes. Absorption of CDOM samples was recorded against Milli-Q water at 443 nm, and internal backscattering was corrected by subtracting the absorbance at 700 nm (Ortega-Retuerta et al. 2009). The dimensionless absorbance (A) was converted to an absorption coefficient (a, m^{-1}) according to Yuan et al. (2007). We selected 443 nm as a reference wavelength because it is the reference wavelength for satellite CDOM determinations.

Calculations and statistical analyses

CDOM turnover time was calculated by dividing ambient a_{443} by a_{443} increases each day. The significance of treatment effects in the enrichment experi-

ments was assessed by ANOVA followed by a comparison of means (*t*-test). Error bars for the bioassay represent a pooled sample standard deviation of the means. All statistical analyses were performed using SPSS software (IBM); results were considered significant at $p < 0.05$.

RESULTS

Ambient temperature, phytoplankton biomass and CDOM

SST at SYB and HKEW during the summer of 2011 were ca. 30 and 28°C, respectively, while salinities were 32 and 33 (Table 1, Fig. 2A). Surface salinity was higher and SST was lower at HKEW, suggesting that upwelling was present (Fig. 2A). Although the SST indicated that there was strong upwelling along the northeastern and southwestern coast of Hainan Island, no upwelling was observed at SYB (Fig. 2A).

Our field measurements and satellite data showed that the chl *a* concentration was ~7 mg m^{-3} at HKEW, which was higher than the 1 to 3 mg m^{-3} at SYB (Table 1, Fig. 3A,B). In contrast, CDOM light absorption at 443 nm was ~0.1 m^{-1} at HKEW, which was lower than at SYB (~0.3 m^{-1}) (Fig. 3C,D). In contrast to the PRE, vertical profiles of salinity and temperature showed less effects of stratification at HKEW (Fig. 4), likely due to the mixing of upwelled water. High salinity and low temperature at the surface indicated a strong upwelling effect at HKEW (Fig. 4). CDOM was relatively low at HKEW in comparison with that in the PRE at both the surface and bottom.

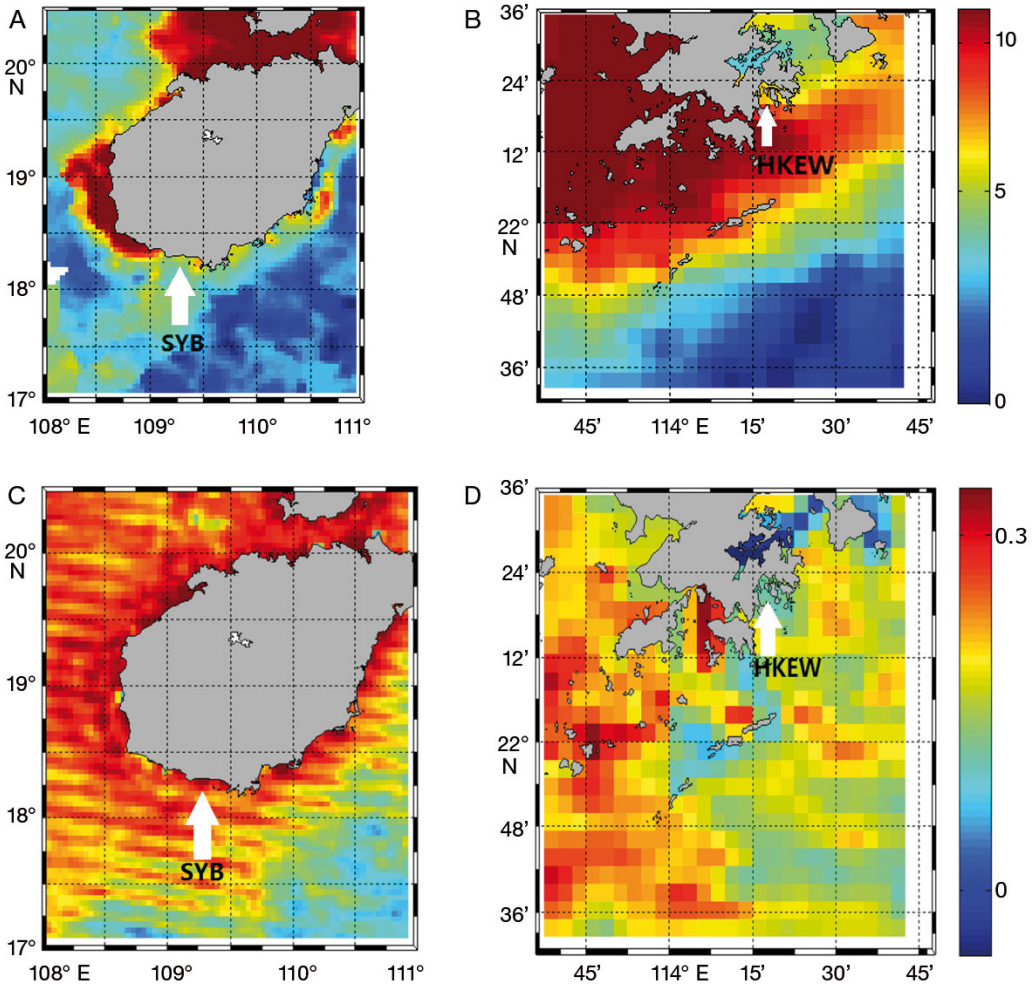
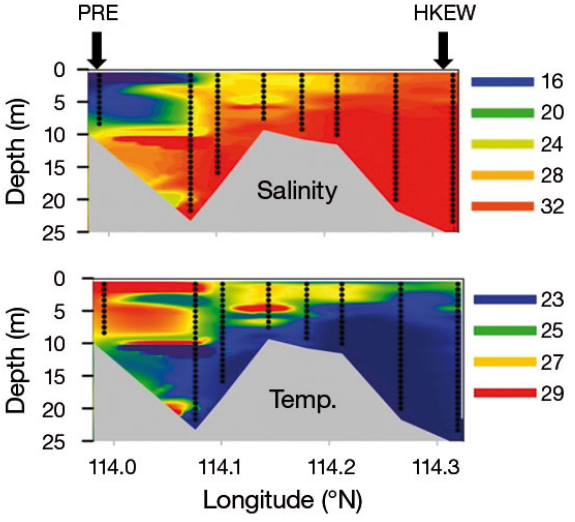


Fig. 3. (A,B) Seasonal (July to September) mean chlorophyll concentration (mg m^{-3}) and (C,D) chromophoric dissolved organic matter light absorption at 443 nm (a_{443}) in Sanya Bay (SYB) and Hong Kong eastern waters (HKEW), respectively, during the summer of 2011. Experimental sites are noted with the white arrows



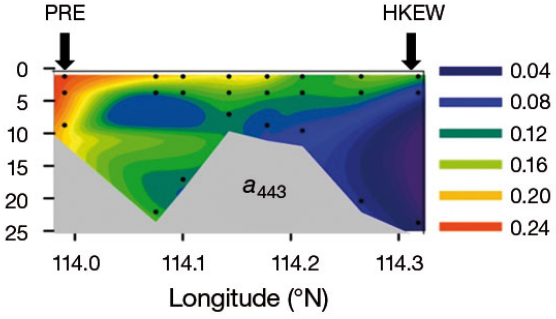
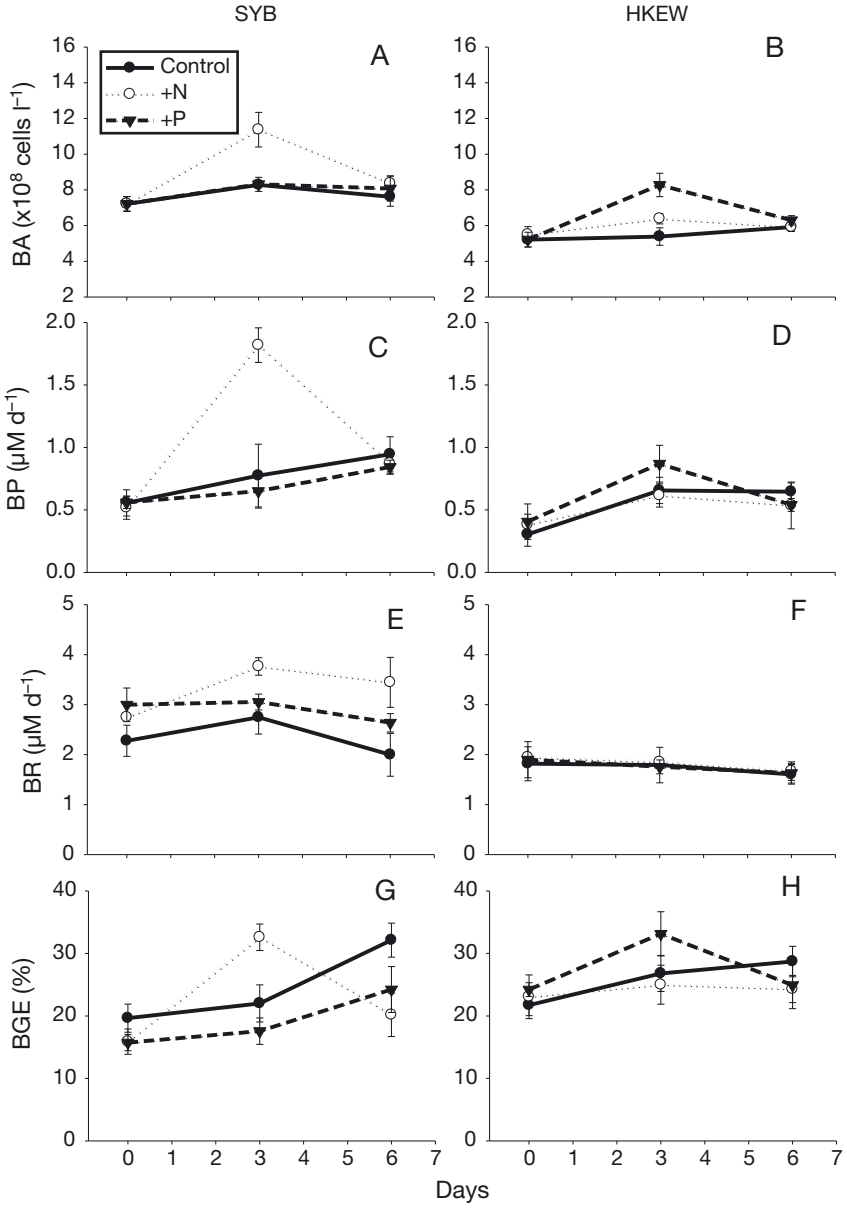


Fig. 4. Vertical contours of salinity, temperature (°C), and light absorption (a_{443}, ml^{-1}) from the Pearl River estuary to coastal/shelf waters in the summer (July 2006). Source: Yuan et al. (2011b) — salinity and temperature data; Yuan et al. (unpubl. data) — light absorption



BA, BP and BR during a nutrient addition incubation

BA was higher at the beginning of the incubation at the SYB site (7 × 10^8 cells l^{-1}) than at HKEW (5 × 10^8 cells l^{-1}) (Fig. 5A,B). Similarly, initial BP and BR were also ~1 to 2 times higher at SYB than HKEW. The N addition (+N) treatment increased BA, BP and BR by 1 to 3 fold after 3 d at SYB ($p < 0.05$), but bacterial growth and BR decreased on Day 6. The P addition (+P) treatment did not significantly affect bacterial growth and metabolism at SYB ($p > 0.05$). At HKEW, BA, BP, and BR did not significantly respond to the +N treatment during the whole incubation period ($p > 0.05$), whereas phosphorus enrichment increased BA and BP after 3 d (Fig. 5B,D).

Fig. 5. Variations in bacterial abundance (BA), production (BP), respiration (BR) and growth efficiency (BGE) with and without (control) the addition of nitrogen and phosphorus in (A,C,E,G) Sanya Bay waters (SYB) and (B,D,F,H) Hong Kong eastern waters (HKEW). Error bars: ±1 SD; n = 3

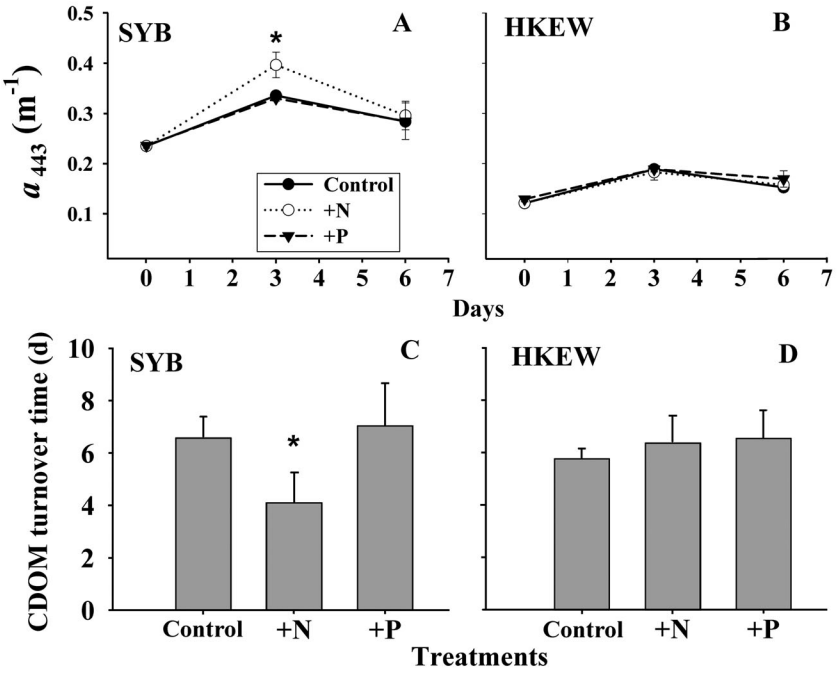


Fig. 6. Variations in (A,B) light absorption at 443 nm (a_{443}), and (C,D) turnover time of chromophoric dissolved organic matter (CDOM) during the incubation experiment with and without (control) the addition of nitrogen and phosphorus at (A,C) Sanya Bay waters (SYB) and (B,D) Hong Kong eastern waters (HKEW). Error bars: ±1 SD; n = 3. Asterisks: significant differences between +N and control samples

Table 1. Surface values of temperature, salinity, dissolved inorganic nitrogen and phosphorus (DIN and DIP), dissolved organic carbon (DOC, ±SD), chl *a*, and chromophoric dissolved organic matter (CDOM) in Sanya Bay (SYB) and Hong Kong eastern waters (HKEW)

Site	Temp. (°C)	Salinity	DIN (µmol l^{-1})	DIP (µmol l^{-1})	DIN: DIP	DOC (µmol l^{-1})	Chl *a* (mg m^{-3})	CDOM (m^{-1})
SYB	30	32	2.0	0.23	9	150 ± 10	2	0.22
HKEW	28	33	4	0.20	20	110 ± 11	6.5	0.13

CDOM light absorption during incubation

Initial light absorption (a_{443}) was ~2 times higher at SYB than HKEW (Fig. 6A,B). After the 3 d incubation at SYB, a_{443} in the control culture increased by ~0.1 m^{-1} (from 0.22 to 0.31 m^{-1}; $p < 0.05$) (Fig. 6A), which was higher than the increase of 0.06 m^{-1} at HKEW ($p < 0.05$) (Fig. 6B). The +N treatment increased a_{443} by 0.16 m^{-1} after the 3 d incubation at SYB compared to initial values, resulting in higher a_{443} than the control ($p < 0.05$) (Fig. 6A). However, no significant differences were observed between other treatment and control samples.

CDOM turnover time by bacteria was ~5 to 7 d in control samples in the first 3 d incubation. Although the increase in a_{443} was higher at SYB than HKEW (Fig. 6A,B), CDOM turnover time exhibited no significant difference due to low ambient CDOM at SYB and HKEW ($p > 0.05$) (Fig. 6C,D). The +N treatment shortened the turnover time to ~4 d at HKEW ($p < 0.05$), while the +P treatment did not significantly alter CDOM turnover time in either of the coastal waters ($p > 0.05$). ΔCDOM (a_{443}) significantly correlated with ΔBA, ΔBP and ΔBR ($R^2 = 0.5$, 0.5 and 0.7, respectively) at SYB and HKEW (Fig. 7), but no significant relationship was found between a_{443} and BGE (data not shown).

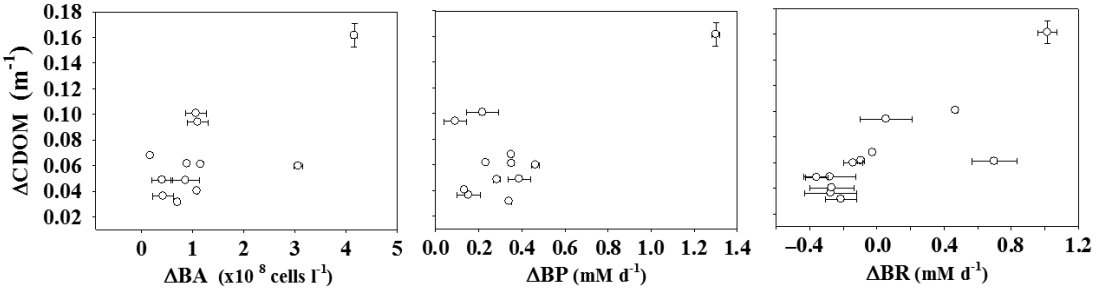


Fig. 7. Relationship between light absorption (Δa_{443}) and (A) bacterial abundance (ΔBA; $R^2 = 0.5$, $p < 0.05$, n = 12), (B) production (ΔBP; $R^2 = 0.5$, $p < 0.05$, n = 12) and (C) respiration (ΔBR; $R^2 = 0.7$, $p < 0.05$, n = 12) in Sanya Bay waters (SYB) and Hong Kong eastern waters (HKEW) (data pooled); the Δ symbol indicates variations from the initial values during incubation. Error bars are SD

DISCUSSION

CDOM and chl *a* distribution

Salinities were ~32 to 33 at the 2 coastal sites we studied (Table 1); hence riverine inputs apparently did not influence salinity levels. Riverine DOM may not contribute greatly to the CDOM distribution in these two areas, but the influence of riverine CDOM might accumulate if CDOM was longer-lived than salinity gradients. In addition, photosynthetically derived DOM did not have a dominant influence on the CDOM distribution, since our data showed that CDOM was higher at SYB than HKEW, while chl *a* exhibited the opposite pattern (Fig. 3). This decoupling of CDOM and chl *a* suggests that the regulating mechanism of CDOM differs between these 2 coastal waters.

CDOM is believed to be a direct result of primary production, and thus should be correlated with chlorophyll concentration (Morel & Maritorena 2001, Romera-Castillo et al. 2010). Indeed, Siegel et al. (2005) reported that the surface patterns of CDOM and chlorophyll concentration were correlated on a temporal basis at the Bermuda Atlantic Time-Series Study (BATS) site. However, additional factors that regulate CDOM should be considered. First, physical processes such as upwellings can affect CDOM sources and concentrations (Day & Faloona 2009). CDOM is usually deficient in deep water, and hence in recently upwelled waters CDOM is lower at the surface than in the surrounding waters (Day & Faloona 2009). Thus, the low CDOM at HKEW may be associated with recent upwelling of CDOM-deficient deep water. In addition to CDOM, red tides occur less frequently due to upwelling in summer along the coast near the PRE; Yin (2003) reported that the southwest monsoon winds result in upwelling, which caused an increase in water circulation and correspondingly fewer red tides in the summer. Thus, while upwelling may indirectly increase CDOM by enhancing production by phytoplankton and bacteria as a result of nutrient increases, it may also decrease CDOM by bringing CDOM-deficient deep water up to the surface. This could explain the contradictory observation reported by Nelson & Siegel (2013), who suggested that CDOM levels were high due to upwelling along the southeast coast of Vietnam in summer and the northwest coast of Luzon in winter. In general, higher values are found in regions of persistent upwelling (e.g. equatorial divergence) (Del Giorgio et al. 2011). CDOM could be low in recently upwelled waters such as at HKEW, where upwelling only occurs during summer in the northern SCS (Harrison et al. 2008).

A number of biological processes can also regulate the concentration of CDOM in the water column, including phytoplankton release, products of zooplankton grazing, bacterial release and uptake and viral interactions (Nelson & Siegel 2002). Although direct production of CDOM components by phytoplankton is known for certain species (Steinberg et al. 2004), Romera-Castillo et al. (2010) suggested that microbial degradation of organic matter is responsible for the majority of autochthonous CDOM production in the Sargasso Sea. In addition, photobleaching, the loss of absorption by CDOM due to light exposure, is believed to be the primary sink for marine CDOM in the samples from the Pacific, Atlantic, Indian and Southern oceans (Swan et al. 2012). Although solar radiation in the SCS is stronger than that in temperate zones because of the low zenith angles (Yuan et al. 2007), few studies have investigated the effects of photobleaching on CDOM in this area. Surface CDOM is likely impacted by photobleaching in our studied waters, and therefore deserves further investigation.

Upwelling and bacterial activities have a considerable effect on CO_2, oxygen, and nutrient distribution in Hong Kong waters (Yuan et al. 2011a). BR accounted for 70 to 90% of community respiration in the coastal waters of Hong Kong (Yuan et al. 2011a), and bacterial excretion was suggested to be an important source of CDOM. Hence, it is reasonable to speculate that upwelling and bacterial activities may also influence the CDOM distribution in our study area, as we discuss below.

Effects of increasing nutrients on bacterial growth

Nitrogen addition enhanced BP and BR before the first 3 d at SYB, indicating that bacteria were N-deficient at SYB. However, the DIN:DIP molar ratios in the ambient seawater (9:1 at SYB, see Table 1) were higher than the N:P ratios of heterotrophic bacteria (3 to 6:1) (Kirchman 2000), suggesting that bacteria might assimilate phosphorus from other sources (i.e. organic phosphorus). In addition to BP and BR, BGE also increased by Day 3 for the N addition treatment in the SYB water ($p < 0.05$) (Fig. 5G,H), suggesting that BP was generally more affected by inorganic NO_3 additions than BR (Alonso-Sáez et al. 2007). The fact that N is the primary limiting nutrient for BP and

BGE has also been found in the Atlantic Ocean and some oligotrophic to eutrophic lakes (Kuipers et al. 2000, Alonso-Sáez et al. 2007).

P deficiency in bacteria was also implicated at HKEW, since P addition enhanced BA and BP (Fig. 5B,D). This is consistent with the results of Yuan et al. (2011c), who reported that P addition increased bacterial oxygen consumption in Hong Kong southern waters. At HKEW, the high DIN:DIP ratio is likely due to upwelling, as inorganic and organic N:P ratios were 10:1 (DIN:DIP) and 28:1 (DON:DOP) at the surface, increasing to 14:1 and >40:1 below 200 m in a non-upwelling offshore region of the SCS (Wu et al. 2003). Since there is continuous pumping of inorganic and organic nutrients into the euphotic zone in upwelling areas, the ambient DIN:DIP ratios in the surface waters were influenced by deep, upwelled water. This explains the relatively higher inorganic DIN:DIP ratios at HKEW than SYB. As a result of the high DIN:DIP ratio (Table 1), bacterial growth would be P-limited in surface upwelled waters of HKEW. The heterotrophic activity of bacteria and the rate of oxygen consumption could be limited due to the higher phosphorus requirements of bacteria relative to that of phytoplankton (Yuan et al. 2011a). Bacteria are rich in phosphorus compared to phytoplankton, and the N:P ratios of phytoplankton biomass are generally about 16:1, much higher than those of heterotrophic bacteria (ca. 3 to 6:1; Kirchman 2000). Phosphorus limitation of bacterial growth was also reported by Sebastián et al. (2004) in the North African upwelling area, where turnover rates of phosphorus were higher than outside upwelled waters, and the enzymatic activity of alkaline phosphatase in upwelled waters appeared to be mainly generated by heterotrophic bacteria.

CDOM variation and its implications

The light absorption of CDOM was much higher at SYB (a_{443} = 0.2 m^{-1}) than at HKEW (0.1 m^{-1}) (Fig. 6). No significant difference in CDOM turnover time was found between SYB and HKEW ($p > 0.05$). Nevertheless, our results showed that bacteria can generate significant amounts of CDOM over a short time scale. In addition, Fig. 7 shows that ΔCDOM variation was significantly correlated with ΔBA, ΔBP and ΔBR ($p < 0.05$), but not ΔBGE (data not shown), suggesting that bacterial growth and metabolism regulated CDOM generation.

Our results confirmed previous studies that bacteria can generate CDOM, as CDOM light absorption increased by 1 to 2-fold on Day 3. BA, BP and CDOM absorption (a_{443}) decreased sharply from Day 3 to Day 6 (Figs. 5 & 6), suggesting that seawater substrate was likely depleted and CDOM might also be consumed by bacteria. CDOM generation and decomposition was coincident with the transition from exponential to stationary growth of bacteria (Huang et al. 2003, Kramer & Herndl 2004, Nelson et al. 2004, Ortega-Retuerta et al. 2009). Experiments by Ogawa et al. (2001) demonstrated that heterotrophic bacteria can rapidly consume labile compounds (glucose or glutamate) and release DOM, and that these processes are linked to cell division during exponential growth. Hence, bacteria play a dual role in the cycling of CDOM (Fig. 1A), since they could be a source of CDOM by exuding it metabolically and also a sink of CDOM by re-mineralizing it (Nelson et al. 2004).

ΔBA, ΔBP and ΔBR were closely correlated with ΔCDOM variations (Fig. 7); thus, variations in nutrients can affect ΔCDOM distribution by regulating bacterial growth and metabolism. In our experiment, the addition of inorganic nitrogen resulted in higher CDOM light absorption at 443 nm after a 3 d incubation at SYB. This is consistent with the results of Huang et al. (2003), who showed that a N addition produced a net increase in the CDOM pool with the presence of microbes during dark incubations in estuarine waters. In addition, inorganic nitrogen can influence the CDOM pool and promote the microbial processes, transforming CDOM into humic substances (Hedges et al. 2000). In contrast, phosphorus enrichment increased BA and BP at HKEW, likely due to the high ambient DIN:DIP ratio resulting from upwelling. Interestingly, N enrichment increased both BP and CDOM generation at SYB, but phosphorus enrichment did not change CDOM in either area, although phosphorus enrichment increased bacterial growth.

In summary, this study showed that bacterial production is an important source of CDOM at HKEW and SYB. In addition, our nutrient addition experiments showed that nutrient variations, especially nitrogen addition, affect bacterial growth and CDOM distributions. Hence, coastal upwelling should be an important factor regulating CDOM levels, when the upwelled waters result in deficient CDOM and high DIN:DIP ratios at the surface. Since nearly 50% of the total light absorption at 400 nm and more than 70% of the total light absorption at 300 nm are due to CDOM (Nelson & Siegel 2013), the increased levels of CDOM could further influence light penetration and phytoplankton photosynthesis.

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