Deep-Sea Research II 117 (2015) 65–85

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Controls on temporal and spatial variations of phytoplankton pigment distribution in the Northern South China Sea

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ARTICLE INFO

Available online 29 May 2015

Keywords:
South China Sea
Pigment
Phytoplankton
Community structure

ABSTRACT

The seasonal and spatial variations of phytoplankton pigment distribution and their interaction with environmental controlling parameters were investigated in the Northern South China Sea (NSCS) at 37 stations covering the coastal region, the continental shelf, the slope, and the deep-water basin during two summer and two winter cruises from 2010 to 2012. Strong spatial, interseasonal, and intraseasonal variations of pigment distribution were observed in the diverse biogeochemical regions, exhibiting extremely dynamic phytoplankton community structure in the NSCS. In addition to chlorophyll *a*, the major pigments observed included divinyl chlorophyll *a* (DV Chl *a*), total chlorophyll *b* (Chl *b*), zeaxanthin (Zea), 19′-hexanoyloxyfucoxanthin (Hex), 19′-but-fucoxanthin (But), fucoxanthin (Fuco), and prasinoxanthin (Pras). Overall, Fuco, Chl *b*, Zea, Hex, But, and Pras were the dominant pigments in the coastal and shelf region; DV Chl *a*, Chl *b*, Hex, But, Zea, and Fuco were the major pigments in the offshore water. After analyzing marker pigment correlations and cellular pigment concentrations, we conclude that Zea, Hex (with But), and Fuco can be used as specific marker pigments for *Synechococcus*, coccolithophores, and diatoms in the NSCS, respectively. We have also derived that prasinophytes accounted for most of the elevated Chl *b* observed in the coastal water and appeared to be an important pico-eukaryotic group (Pico) in the coastal region. With varying cellular pigment concentrations observed vertically and seasonally, it is essential to separate sampling stations to different biogeochemical domains to apply pigment to Chl *a* ratios to estimate phytoplankton community structure in the NSCS. We observed close association between soluble reactive phosphate and Chl *a* abundance in coastal, shelf, and deep-water regions during summer cruises. Major nutrient supply appears to be the main controlling factor on the temporal and spatial variations of major pigment distribution. The supply is mainly driven by terrestrial input in summer and water mixing strength in winter. During the summer periods, major nutrients are primarily supplied from riverine and groundwater discharge; during winter periods, strong winter monsoon intensifies sub-surface water mixing and results in nutrient and pigment elevation in the euphotic zone. Also driven by the monsoon, the intrusion of the low-temperature coastal current from Taiwan Strait to the coastal region of the NSCS may replace or alter the phytoplankton community in the coastal region in winter. Overall, the temporal and spatial variations of phytoplankton pigment distribution in the NSCS are regulated by the fluctuations of these two major environmental forcings.

1. Introduction

Phytoplankton plays a central role for material cycling through the biological pump in the ocean. Understanding phytoplankton abundance and community structure is critical for quantifying and understanding how biologically active material is cycled and transported in the ocean. Both phytoplankton common pigments and phylum specific marker pigments are useful parameters to estimate total phytoplankton biomass and their community structure in the ocean. For example, chlorophyll *a* (Chl *a*) has been widely used to estimate total phytoplankton biomass and primary production in the ocean (Behrenfeld and Falkowski, 1997). The concentrations of some specific marker pigments may be linearly correlated with the biomass of their corresponding phytoplankton groups. For example, divinyl chlorophyll *a* (DV Chl *a*), a unique pigment for *Prochloroccocus* (*Pro*), is used to estimate the abundance and the distribution of *Pro* when the ratio of marker pigment to total Chl *a* is known (Goericke and Repeta, 1993). However, some marker pigments exist in several different phytoplankton groups. For example, Zea exists in *Synechococcus* (*Syn*),

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http://dx.doi.org/10.1016/j.dsr2.2015.05.015
0967-0645/

prasiophytes, and chlorophytes; Chl *b* exists in prasiophytes, chlorophytes, and englenophytes. Numerical computer programs, such as CHEMTAX, have thus been developed to utilize multiple pigment markers and factor analysis to assess the relative contribution of major phytoplankton classes to total Chl *a* concentrations and to quantify relative species percentages in oceanic water (Mackey et al., 1996; Latasa, 2007).

Since different phytoplankton groups possess different marker pigments and ecological niches, both chemical and physical parameters of environmental conditions concurrently influence the distribution of phytoplankton's marker pigment composition in oceanic waters. For example, cyanobacteria, including *Pro* and *Syn*, possess unique ecological niche to survive in oligotrophic waters; dinoflagellates and diatoms are able to dominate in relatively eutrophic water. Understanding the influence of environmental control on the spatial and temporal variations of phytoplankton pigment distribution would provide essential information to utilize pigment composition to quantify community structure and its response to environmental changes. This kind of study may be particularly challenging in marginal seas, where both environmental conditions and phytoplankton community structure vary extensively with time and location. The Northern South China Sea (NSCS), one of the largest marginal seas in the world, is a biogeochemically extremely dynamic oceanic province both temporally and spatially. The biogeochemistry of this tropical sea is driven by diverse anthropogenic and natural forcings at strengths that are among the strongest in the world (Wong et al., 2015). The major forcings include large riverine and submarine groundwater discharges with high nutrient loading (Liu et al., 2012), high atmospheric deposition with material from multiple natural and anthropogenic origins (Ho et al., 2010), different seasonal monsoons with varying directions and strengths, typhoons occurring with high frequencies and strengths, frequently occurring cold and warm eddies, internal waves with the highest amplitudes in the world, and the intrusion of the western boundary current, the Kuroshio water.

Several previous studies have reported the temporal and spatial variations of pigment and phytoplankton community information in the NSCS (e.g., Ning et al., 2005; Cai et al., 2007; Chen et al., 2011; Huang et al., 2010). Most of the published studies focus on investigating the abundance of *Pro*, *Syn*, and Pico by pigment analysis or using flow cytometer in a limited region of the NSCS. Chen et al. (2011) reported seasonal variation patterns for *Pro*, *Syn*, and Pico in the NSCS on one summer and one winter cruises. The study by Zhai et al. (2011) investigated phytoplankton pigment patterns and reported the community composition by using CHEMTAX near the Pearl River in February 2009. Huang et al. (2010) found that different origins and ages of eddies are important processes influencing the variations of phytoplankton community in the NSCS. Using a multiple parameter approach by combining data obtained from satellite images, pigment concentrations, and phytoplankton abundance, Pan et al. (2013) established algorithms to derive the temporal patterns of phytoplankton community structure in the NSCS. Although these studies have provided valuable information for phytoplankton community structure in the NSCS, the temporal and spatial variations of the community structure in the NSCS as a whole still largely remains to be explored. In addition, comprehensive studies examining how chemical and physical factors interact with the variations of pigment distribution and phytoplankton community structure are also limited.

Here, we have determined the pigment composition in the euphotic zone of the NSCS for four cruises within three years, covering the coastal region, the continental shelf and slope, and the deep water basin. We have taken the advantage of this thematic study (NoSoCs) where many biogeochemical parameters were measured simultaneously, including major nutrient concentrations, basic hydrographic parameters, the abundance of *Pro*, *Syn*, and Pico, and temporal variations of climatology (Wong et al., 2015; Pan et al., 2015) to examine the environmental controls on the temporal and spatial variations of pigment composition and phytoplankton community structure in the region.

2. Material and methods

2.1. Sampling time and station

Pigment samples were collected during the following four cruises, June 2010, December 2010 to January 2011, December 2011, and late August to early September 2012. Detailed information of the sampling dates and time are shown in Table 1. Following the depth contour lines, the sampling stations are separated into four different coastal to offshore water transects numbered from the northeastern to the southwestern ends of the NSCS. The stations in order from offshore to inshore stations from T1 to T4 transects are 2 to 12, 25 to 13, 54 to 41, and 26 to 40, respectively (Fig. 1). The first cruise carried out in June 2010 covered all four transects. Due to the limitation of sampling time and severe weather in winter, the sampling stations of the 2nd cruise carried out in December 2010 were mainly located at T4 and the other four stations at T1 (Table 1). The cruises carried out in 2011 and 2012 were focused at T3, expending additional resources to study pigment distribution at a time series station, SEATS (Table 1). Samples were taken at SEATS for more than 30 h to study diurnal variations of pigment concentrations during the two cruises in 2011 and 2012 (Table 1).

2.2. Pigment sampling and analysis

Seawater samples for pigment analysis were collected by Go-Flo bottles mounted on a CTD rosette during every cruise. Two liters of seawater were transferred from Go-Flo bottles to 2.5 L dark bottles. Right after collection, the seawater samples were filtered on board through pre-combusted 47 mm Whatman GF/F filters under vacuum pressure of less than 100 mm Hg. The filters were preserved in tissue embedding cassettes and were kept frozen in liquid nitrogen until further processing in our land-based laboratory. In the land-based laboratory, the filters were first processed through freeze dryer for 24 h to remove water. Then the pigments in the filters were extracted by 90% acetone in the dark with sonication in an ice slurry for one hour. The extracted pigment samples were then filtered through a 0.2 μm PTFE syringe filter to remove particulate debris. The filtered extracted pigment samples were then ready to be analyzed. The pigments identified in the samples include mono-chlorophyll *a* (Chl *a*), fucoxanthin (Fuco), prasinoxanthin (Pras), alloxanthin (Allo), peridinin (Per), divinyl chlorophyll *a* (DV Chl *a*), chlorophyll b (Chl *b*), zeaxanthin (Zea), 19′•-hexanoyloxyfucoxanthin (Hex), 19′-but-fucoxanthin (But), chlorophyll *c2* (Chl *c2*), chlorophyll *c3* (Chl *c3*), lutein (Lut), neoxanthin (Neo), violaxanthin (Vio), diadinoxanthin (Diad), carotenes (Car), and diatoxanthin (Diat). Unabbreviated pigment names are listed in Table 2. The standards of Chl *a* and Chl *b* were purchased from Sigma Chemicals, and all other pigments were purchased from DHI (Denmark). The accuracy of the concentrations of Chl *a* and Chl *b* standards were periodically validated by UV–vis spectrophotometer (Shimatzu 1700) and fluorescence spectrophotometer (Hitachi F-7000).

We used both HPLC (Shimadzu LC-10A, Japan) and UPLC (Waters, ACQUITY, US) techniques for pigment analysis. The HPLC method was equipped with C18 column (Tosoh, 250×4.6 mm^2, particle size of 5 μm) by using ternary gradient elution procedures