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Characteristics of phytoplankton communities and their biomass variation in a gas hydrate drilling area in the northern South China Sea

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ABSTRACT

We analyzed the data obtained from field observations on a gas hydrate drilling area in Dongsha of northern South China Sea (SCS) in middle May (before drilling) and early October (after drilling) in 2013. The variation in the phytoplankton communities and biomass as well as the impacts of environmental factors including dissolved methane was studied. Results indicated that the gas hydrate drilling area in Dongsha, SCS exhibited a typical low-nutrients low-chlorophyll *a* (LNLC) environment accompanied with low phytoplankton abundance. A total of 103 taxa belonging to 52 genera of 5 classes were identified, with diatoms and dinoflagellates dominating the community. Both phytoplankton abundance and chlorophyll *a* (Chl *a*) were highest at the subsurface maximum layer. The subsurface chlorophyll maximum (SCM) before and after drilling were stabilized at 75 m (0.30 ± 0.06 mg/m^3 and 0.51 ± 0.29 mg/m^3, respectively), while the subsurface maximum of abundance after drilling went deeper to 75 m (604.17 ± 313.22 cells/L) from the surface (707.14 ± 243.98 cells/L) before drilling. After drilling, phosphate and Chl *a* increased significantly, but no significant differences were observed on abundance. Dominant species of diatoms were basically constant with dinoflagellates becoming more apparent in higher occurrence and abundance, while Cyanophyta was diverse after drilling. Redundancy analysis (RDA) and Spearman's correlation analysis both indicated that temperature, pH and phosphates were major factors causing fluctuation in phytoplankton community structure, while dissolved methane had non-significant impact directly. We clearly found both abundance and Chl *a* increased in particular water layers (between 50 and 75 m) and at stations (DS06, DS08 and DS15) where dissolved methane concentrations were also abnormally high. This study appeared to partly coincide with the findings of natural oil seeps in the Gulf of Mexico, which assumed that the turbulence from the natural oil and gas leaking zone could raise the bottom water through the rising bubbles and bring cold nutrient rich waters to the thermocline from the deep seeps. This plume-generated upwelling could then fuel a bottom-up effect on the photosynthetic species in the upper pelagic waters within the euphotic zone.

1. Introduction

Gas hydrate is an ice-like crystalline compound with clathrate structure and composed of natural gas, mainly hydrocarbons and water molecules, which are formed under low temperature and high-pressure conditions (Sloan, 1998). In the marine environment, gas hydrates are generally observed in deep-water slope habitats at depths over 300 m. The same sites are also important natural sources of atmospheric methane, a greenhouse gas that has significant implications to global climate changes and feedback effects to the earth's climate system (Hesselbo et al., 2000). Seabed gas hydrates decompose and release methane gas through several mechanisms including formation of typical seabed cold springs. In most deep-sea vents however, methane is consumed by marine microorganisms during its release, which is then transformed into carbon dioxide (CO_2). This excess CO_2 would lead to decrease in pH of the surrounding waters, and together with lower oxygen levels, would be extremely unfavorable for the plankton (Bala et al., 2005; Shakhova et al., 2014). Several studies showed that increase in the concentrations of dissolved methane and carbon dioxide in sea water were coupled by the simultaneous increase in the photosynthetic efficiency of marine phytoplankton, resulting in short-term higher phytoplankton abundance and biomass (Fleeger et al., 2003; Solomon et al., 2007; D'Souza et al., 2016). However, long-term continuous accumulation of CO_2 could lead to acidification, and such

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corrosive conditions would seriously threaten the survival of many marine organisms including phytoplankton (Hönisch et al., 2012; Hesselbo et al., 2000).

The Dongsha Islands, 315 km from the Zhujiang (Pearl River) Estuary, are located in the northern continental slope of the South China Sea (SCS). These islands also have the least coverage of reefs. In summer of 2004, SO177 Sino-German joint voyage found a cold-carbonate seep area, which was named Jiulong Methane Reef, with a total area of about 430 km^2 in the northeastern waters of Dongsha Islands. It is currently the largest authigenic carbonate area in the world. The chemoautotrophic communities live around the methane reef indicating a cold-seep nozzle, including bivalves, tubular worms and methanotrophic bacteria (Suess and Sonne, 2005). The presence of these species indicates large-scale methane release activities, which might be related to hydrate decomposition that have occurred during in geological periods in the northern slope of the SCS, with the cold seep still manifesting some minor activities (Suess and Sonne, 2005; Zhang et al., 2014).

Geological activities such as petroleum leakage and hydrothermal vents occurring in the seabed at the edge of the continental shelf greatly facilitated the productivity of the seabed organisms (Hovland et al., 2012). The decomposition of seabed gas hydrates also supported the local higher reproduction of the benthos community (Leblond et al., 2014). However, little is known if they also had effects on the phytoplankton community, including their community structure, biomass and primary productivity in the upper waters (D'Souza et al., 2016). Hypothesis suggested the turbulence from the natural oil or gas leaking zone could raise the bottom water through the rising bubbles and bring cold nutrient rich waters to the thermocline from the deep seeps. This plume-generated upwelling could then fuel a bottom-up effect on the photosynthetic species in the upper pelagic waters within the euphotic zone, similar to the influence of eddy currents driving up-flows in the subtropical waters, bringing rich nutrients that affect phytoplankton biomass and productivity (Sauter et al., 2006; McGillicuddy et al., 2007; Leifer et al., 2009; D'Souza et al., 2016).

Previous studies on the gas hydrates in the SCS were more focused on their resource potential and exploration, with some studies were on the benthos and associated microorganisms such as the oxygen and carbon isotopes in foraminifera shells (Yu et al., 2008; Jiang et al., 2005), tubular worm shells (Chen et al., 2005; Lin et al., 2007) and methanogenic bacteria (Su et al., 2007). However, responses and feedbacks of the communities in the upper waters, especially the plankton in the euphotic layer to the dissolved methane are less studied (e.g. Leifer et al., 2009; D'Souza et al., 2016). As research continues into marine gas hydrate exploration, the response and underlying mechanisms of the pelagic ecosystem exposed to seabed methane seepage urgently needs to be recognized and further studied.

In this study, through the continuous sampling of the waters from the gas hydrate drilling area in Dongsha of the northeast slope of the SCS, the effects of the dissolved methane released through the decomposition of gas hydrates on the marine phytoplankton communities were assessed. We specifically monitored phytoplankton abundance, Chl *a*, species composition and their correlation to the dissolved methane. We intended to look for evidence on how the phytoplankton communities and biomass were affected by dissolved methane released from the seabed in SCS, so as to support to previous hypothesis.

2. Materials and methods

2.1. The study area and station setting

The study area was located near the central uplifted area of the basin southwest of Taiwan, named the Dongsha slope, west towards Taiwan canyon and east to Penghu canyon (Fig. 1). The study sites have complex submarine landforms, including a trough, sea valley, sea mountain, escarpment, slope, scourway and seaknoll. Drilling in this area was carried out in July 2013. Meanwhile, phytoplankton samples were also collected in Dongsha gas hydrate area aboard the SRV "Haiyang IV" in middle May (before drilling) and early October (after drilling) of 2013. All stations used the position of the drilled hydrate wells as the reference as shown in Fig. 1. In middle May 2013, a total of 7 stations were visited, and were revisited in early October 2013. In July 2013, the gas hydrate chemical samples were all obtained in stations DS06, DS08 and DS15 during the drilling period (Zhang et al., 2014).

2.2. Sampling and determination

Temperature and salinity data were obtained using the SeaBird Electronics CTD system (SeaBird Electronics, SBE917 Plus). The ROSETTE hydrophore was used for the collection of water samples, drawn from different depths specifically at 0 m, 30 m, 50 m, 75 m, 100 m, 150 m and 200 m. In the laboratory, water samples of phytoplankton were allowed to settle before being concentrated, then the Utermöhl method was conducted to count and identify the cells under the Zeiss Z1 inverted optical microscope (Zeiss, Germany) based on previously described descriptions and taxonomic keys (Jin et al., 1965; Hasle and Syvertsen, 1997; Sun and Liu, 2002; Guo and Qian, 2003). The abundance was calculated for each species and expressed as cells/L. Indistinguishable taxa were further characterized using an electron scanning microscopy (Hitachi, Japan).

Phytoplankton biomass was estimated using Chl *a* concentration as the proxy through spectrofluorometric method according to Yentsch and Menzel (1963). The sampled layers were the same as those collected for microscopy. Environmental parameters including nutrients (silicate, phosphate and ammonium) and pH were measured following the methods described in "Specification of Oceanographic Investigation in China" (GB/T12763.4–2007, General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2008). Silicon molybdenum blue spectrophotometric method was used for silicate, while phospho-molybdenum blue colorimetric method was used for phosphate. Indigo blue spectrophotometric method was used for ammonium. Electrode potentiometric method was use for pH.

The Franatech METS sensitive methane sensor (No. GT196-E399) (the measurement accuracy was 1 nmol/L and the sensitivity was 0.1 nmol/L) combined with CTD was used to determine the dissolved methane in sea water. The power supply of METS and data collection was provided by the CTD system, and the sampling frequency was every 1 s.

2.3. Statistical analysis

Independent-sample *t*-test was selected to evaluate variations in phytoplankton parameters and environmental factors before and after drilling (SPSS17.0 for Windows, Chicago, USA). Spearman's rank correlation coefficient was then used to explore correlations between phytoplankton and environmental factors (SPSS17.0 for Windows, Chicago, USA). The relationships between the biological groups and their corresponding environmental groups were analyzed via canonical correlation analysis (Canoco for Windows 5.0 software). ODV software was cited as http://odv.awi.de, 2009.

3. Results

3.1. More geophysical evidence of gas hydrates in Dongsha, SCS from this study

In this investigation, the obtained geochemical data indicated that the air source of gas hydrates in Dongsha seabed was mainly from the microbiogenic gas according to corresponding author Liang et al. (2017). Moreover, Zhang et al. (2014) documented the logging-while-