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Response of phytoplankton community to nutrient enrichment in the subsurface chlorophyll maximum in Yellow Sea Cold Water Mass

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ABSTRACT

The subsurface chlorophyll maximum (SCM) is a prominent biological feature in stratified waters, and may contribute substantial biomass to the water column. Understanding the transient variations of SCM phytoplankton in response to episodic nutrient input is crucial to accurately estimate integrated primary production and to assess the impact of the perturbation to the pelagic ecosystem. A microcosm experiment designed to investigate the responses of SCM phytoplankton community to pulsed nutrient enrichment was conducted in summer 2011 in the Yellow Sea Cold Water Mass area. During the experiment, the incubation cultures sustained a high photosynthetic yield (F_v/F_m) indicating that the phytoplankton was photosynthetically competent and well acclimated to conditions of irradiance and nutrient supply at the SCM. Both F_v/F_m and Chlorophyll *a* (Chl *a*) responded significantly in P enriched treatments, but not in the N enriched treatments. The largest increase of Chl *a* and F_v/F_m occurred when P and N were added simultaneously. *Synechococcus* abundance decreased sharply during the incubation, while picoeukaryote abundance increased in the P and NP addition treatments. The phytoplankton community shifted from smaller dinoflagellates dominated in the natural environment to larger diatoms dominated under nutrient enrichment conditions. The results indicated that the phytoplankton at the SCM was co-limited by P and N and had a higher requirement for P relative to N. The additional nutrient supply enhanced photosynthetic activity and favored the dominance of larger diatoms which are beneficial to carbon export. Our study suggested that episodic nutrient input induced by various physical processes make a significant impact on the phytoplankton community at the SCM. This information is important for better understanding and predicting biological responses to future climate change.

1. Introduction

The subsurface chlorophyll maximum (SCM, or deep chlorophyll maximum, DCM) is a common and widespread biological phenomenon in stratified waters [5,14]. The SCM community can contribute a substantial part of the annual production to the water column whether in oceanic or shelf and coastal seas, therefore has received considerable attention in field observations [27,30,35], numerical modeling [9,11,14,23] and remote sensing studies [41].

Seen as a relatively stable feature, the SCM can be disturbed by physical processes such as wind and tidal mixing, upwelling, internal waves [19,22,23,36] and episodic climate events (mixing events induced by storms or eddies). Most of these processes are associated with nutrient supply into the upper ocean and can significantly change the phytoplankton biomass, species composition and community succession [4,19,40]. A recent study conducted in the South China Sea showed that Chl *a* blooms can occur not only in the surface layer but also in the subsurface layer after the passage of a typhoon [45]. Increasing numbers of observational and modeling studies demonstrate that DCM can exhibit a long transient response even after a single short perturbation [19], and the related episodic production or phytoplankton accumulation can generate significant carbon export [14,15,29].

Unlike the phytoplankton biomass enhancement in the surface layer, which can be estimated by ocean color data, the biological process in SCM can barely be captured by satellite observations. Because of this, the variation in SCM and the associated changes in phytoplankton community are less well known and rely on field observation (in situ) and experimental approaches.

The Yellow Sea, located between mainland China and the Korean Peninsula, is a productive semi-closed marginal sea of the Pacific Ocean, with depths ranging from 90 m in the central trough to less than 20 m within 50 km off the coast [48]. As a typical temperate continental sea, the Yellow Sea is characterized by marked seasonality under the control of the East Asian Monsoon climate and complex

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hydrodynamics [34]. From late spring to early autumn a combination of strong solar heating and weak wind forces induces strong stratification in the central Yellow Sea [7]. In the upper mixed layer, nutrients are depleted after spring bloom and, beneath the thermocline, the low-temperature and nutrient-rich Yellow Sea Cold Water Mass (YSCWM) is found, the most noticeable phenomenon in the summer bottom layer of the Yellow Sea [18,31].

In the summer stratified central Yellow Sea, the SCM is widely observed yet has only been described briefly in previous studies [10,17, 22,46]. Given the importance of SCM to the function of the pelagic ecosystem, the response of the phytoplankton community to environmental disturbance (particularly nutrient entrainment) deserves further investigation. In the present study, a microcosm nutrient enrichment incubation experiment was conducted at an off-shore station located in the summer stratified central Yellow Sea with aims to: (1) identify which nutrient controls phytoplankton growth; (2) assess the responses of the phytoplankton community to the episodic input of nutrients in terms of photosynthetic performance, Chl *a* concentration, picophytoplankton abundance and shift of microphytoplankton community species composition within the SCM. The results of the study were intended to illustrate the direct biological response in the SCM to pulsed nutrient intrusion induced by episodic events, and help us to better understand and predict the biological response of the SCM to future climate change.

2. Material and method

2.1. Sampling

This research was carried out on board R/V *Xiangyanghong* 8 on 23–27 August 2011 during a Yellow Sea Cold Water Mass (YSCWM) cruise. The experiments were performed at station B3 (36°0.0'N, 124°0.0'E) located offshore in the YSCWM-influenced area (Fig. 1) where the hydrodynamic environment was relatively stable.

The vertical profiles of salinity, temperature and Chl *a* data were collected with a SBE-19-plus CTD unit with additional chlorophyll fluorescence sensor (Sea-Bird Co.). The CTD and additional sensors were calibrated before the cruise. The depth of the SCM was determined by the downward profile of the fluorescence signal.

Water samples were collected with 5 L Niskin bottles attached to a Sea Bird CTD rosette sampler at a depth of 0 m, 5 m, 10 m, SCM, 30 m, and 50 m and bottom to measure the chemical variables of inorganic nutrients (nitrate, silicate and phosphate). Water samples at the SCM layer were collected for the nutrient enrichment experiments.

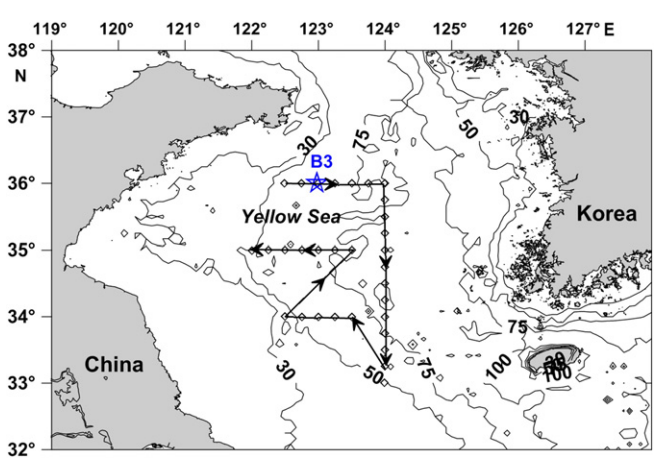

Fig. 1. Survey stations of summer cruise 2011 and the location of B3 for nutrient enrichment experiment.

2.2. Nutrient enrichment experiments

Water samples were prescreened through 200 μm nylon mesh to remove large zooplankton and debris before being dispensed into 12 acid-cleaned 1 L transparent polycarbonate bottles.

Nutrients were added, alone and in combinations: +N, +P, +NP and control, at day 0 in a single pulse, according to Table 1. The concentration and ratio of nutrient added were similar to those in the bottom water of the study area. N was added as potassium nitrate (KNO_3), and P as potassium dihydrogen phosphate (KH_2PO_4). Each treatment was performed in triplicate. Experimental treatments were incubated for 9 days in an on-deck controlled incubator at a temperature of 15 °C and irradiance of 20 μ mol photons m^{-2} s^{-1} measured at SCM and with a light–dark cycle of 12 h:12 h. Responses of phytoplankton to nutrient enrichment were evaluated through changes in biomass (Chl *a*), photosynthetic competency (i.e. maximum photochemical quantum yield of photosystem II = F_v/F_m), abundance of picophytoplankton and species composition of microphytoplankton. The bottles were opened daily and gently shaken twice a day. Sub-samples for in vivo Chl *a* and F_v/F_m measurements were collected from each bottle daily (except for days 6 and 8). Nutrient concentrations, picophytoplankton abundance and phytoplankton species composition were only evaluated at the beginning and end of the experiments. Cultures were sampled at the same time (±30 min) to minimize effects of diel periodicity on algal physiological factors [32].

2.3. Laboratory analyses

2.3.1. Photosynthetic competency and Chl a concentration

The photosynthetic competency (F_v/F_m) of the algae and the Chl *a* concentration were estimated by pulse-amplitude-modulated fluorometry (WALZ Phyto-PAM). Before measurement, triplicates of 2 ml samples were dark-adapted for 15 min.

2.3.2. Nutrient analysis

Background nutrient concentrations at the sampling station and the nutrient concentrations at the beginning and end of the incubation experiment were measured. 500 ml of seawater was filtered through GF/F filters and the filtrate immediately stored at −20 °C until analysis at the land laboratory. Concentrations of nitrate, phosphate and silicate were measured using a nutrient auto-analyzer (SKALAR). The detection limits were 0.1 μmol L^{-1} for nitrate, 0.01 μmol L^{-1} for phosphate and 0.05 μmol L^{-1} for silicate.

2.3.3. Flow cytometry

Cytometric analyses for picophytoplankton were performed with a FACSCalibur (Becton Dickinson) flow cytometer. Populations were differentiated based on their scattering and fluorescence signals [26]. Subsamples (5 ml) were fixed with paraformaldehyde (final concentration 1%) immediately after collection and then freeze-trapped in liquid nitrogen until analysis by flow cytometry in the laboratory [26]. The background fluorescence signal (blank) was measured using 0.2 μm filtered seawater and was subtracted from the measured variable fluorescence.

2.3.4. Microscopy counts

Samples for identification and enumeration of phytoplankton were preserved with 1% acid Lugol's solution immediately after collection,

Table 1
Nutrients added in different experimental treatments.

No.	Treatment	Nutrient added (μmol L^{-1})
1	Control	–
2	+N	10
3	+P	0.6
4	+NP	10 N + 0.6 P