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Newly recorded *Karlodinium veneficum* dinoflagellate blooms in stratified water of the East China Sea

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ABSTRACT

Karlodinium veneficum is a cosmopolitan species, but has been poorly recorded in the East China Sea (ECS) due to its small size and difficulty in identification. The bloom dynamics of this species is not well understood globally. In this study, we examined its morphological characteristics that suggest the *K. veneficum* is the co-occurring bloom causative species of large scale *Prorocentrum donghaiense* blooms in spring 2011. The epicone of *K. veneficum* recorded in the investigated area is conical or rounded, and the hypocone is hemispherically rounded. The ventral pore is located at the left side of the apical groove. Nucleus is positioned centrally within the hypocone. Four large irregular chloroplasts are equally distributed in the epicone and hypocone. The mean length of cultured cells was 13.6 ± 1.2 μm (range 11.0–15.8 μm) and the mean width was 10.0 ± 1.1 μm (range 8.0–12 μm) ($n=50$). Cell abundance of *K. veneficum* population was low, in the region 1000–1600 cells L^{-1}, along a transect in the East China Sea on April 19, 2011, when the water column was not distinctly stratified. Cell densities reached 3×10^7 cells L^{-1} along the same transect on May 13 2011 when the bloom occurred in the 10 m layer surface and the water column was distinctly stratified. Cell abundances therefore appear closely related to water column stratification.

1. Introduction

Increasing harmful algal blooms (HABs) in coastal areas have resulted in ecosystem damage and human health impacts worldwide (Anderson et al., 2008; Heisler et al., 2008). Such problems are often related to two types of HAB: high-biomass producers and toxin producers (Geohab, 2001). Several toxic blooms have recently ocurred in the East China Sea (ECS). *Gymnodinium catenatum* and *Karenia mikimotoi* blooms have been recorded (Zhou et al., 2008a; unpublished data from the State Oceanic Administration People's Republic of China), as well as large-scale high-biomass algal blooms of *Prorocentrum donghaiense* Lu over the last two decades (Lu et al., 2005; Li et al., 2009, 2010). Those toxin producers may lead to fish and shellfish kills, and also impact human health even at low cell abundance (Geohab, 2010).

Karlodinium veneficum (D. Ballantine) J. Larsen is a very small, unarmoured dinoflagellate (Ballantine, 1956; Wang et al. 2011) and has been considered as one of the causative species related to fish killing events (Ballantine, 1956; Place et al., 2008). *K. veneficum* shares the characteristics of the genus of *Karlodinium* with a straight apical groove and distinct ventral pore (Daugbjerg et al., 2000). Blooms of *K. veneficum* were first described in South Africa (Braarud, 1957; Pieter and van der Post, 1967), and later in Europe (Bjornland and Tangen, 1979; Nielsen, 1996), North America (Li et al., 2000; Terlizzi et al., 2000) and Australia (Ajani et al., 2001; Cosgrove et al., 2000). Very recently, this species was recorded in the coastal water near Nanji Island of Zhejiang province, China (Wang et al., 2011). However, *K. veneficum* is only poorly recorded in the ECS, most likely due to its small size and difficulty in identification. The bloom dynamics of this species are not well understood globally. In this study, we examined its morphological characteristics, and suggest that *K. veneficum* is a species which co-occurs with large scale *P. donghaiense* blooms and present its distribution pattern in a stratified water column of the East China Sea in the spring of 2011.

2. Methods

2.1. Study area

This study was conducted in the ECS between March 29 and May 27, 2011 (Fig. 1). Five transects (Ra, Rb, Za, Zb and Zc) between 28°N and 31°N, normal to the coastline, were sampled in the coastal area of the ECS. These trasects crossed both the 20 m and 60 m isobaths.

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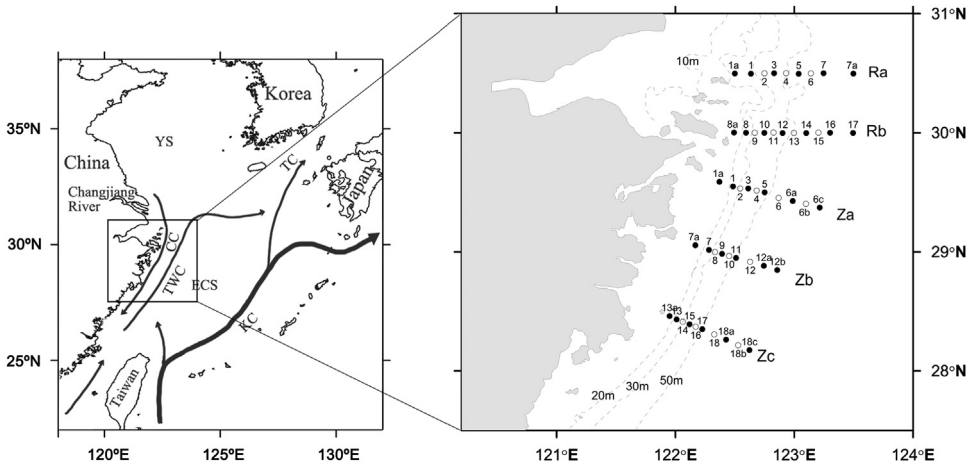

Fig. 1. Sampling stations and circulation pattern in the East China Sea (ECS) (modified from Zhou et al., 2003; Naimiea et al., 2001). Left panel, YS: Yellow Sea; KC: Kuroshio Current; TWC: Taiwan Warm Current; CC: Coastal Current (seasonal current northeastward in summer and southwestward in winter); TC: Tsushima Current. Right panel: closed circles indicate comprehensive stations and open circles indicate hydrological stations, with labels above or below the station symbols, respectively; transect labels are marked to the right of the transects; dotted lines are isobaths (m).

Two main currents, the Coastal Current (CC) and the Taiwan Warm Current (TWC) interact in this area. The direction of the CC varies seasonally with the monsoon (Zhou et al., 2008a). It flows southwestward when north and northeast wind prevails in winter, and turns to flow northeastward when north winds become weaker and southwest winds start to prevail in spring. Both the freshwater plume of the Changjiang River and the TWC become stronger in spring, and the latter invades the lower part of water column, a process which intensifies stratification in the offshore water of the ECS. The TWC has a higher salinity than that in the coastal area, since it originates and extends from the Kuroshio Current (KC). There is an upwelling belt, about 40 km wide, between the 20 m and 50 m isobaths which runs parallel to the Zhejiang coast line (Luo, 1998; Luo and Yu, 1998; Qiao et al., 2006).

2.2. Sample collection

Two types of sampling stations were sampled mainly during daylight hours. Comprehensive stations included hydrological and biological information, whereas hydrological stations included only physical information. An SBE 19plus CTD (SeaBird Electronics Inc., USA) which was interfaced with an in situ fluorometer (WET Labs, WETStar fluorometer, WS1S-1293) was used to profile from the sea surface to the bottom of the water column in order to determine the depth of the chlorophyll maximum layer (CML, also referred to as the middle layer) before sampling at each comprehensive station. Water samples were collected using 30 L Niskin bottles in the surface layer, CML and bottom layer. Extra sampling depths were added at selected stations. Water samples (500 mL) for phytoplankton analysis were transferred into 550 mL polyethylene terephthalate (PET) bottles and were then fixed with 3–5% acidic Lugol's solution. Environmental parameters, such as temperature, salinity, density, dissolved oxygen (DO) and chlorophyll-a (Chl-a) were recorded at every station at 0.5 m depth intervals using the CTD probe.

2.3. Species isolation and culturing

Naturally occurring *K. veneficum* cells were isolated from samples taken within the investigated area during its bloom time in May, 2011. Approximately 1.5 L of sample was screened through a 20 μm plankton net to remove larger plankton cells. Small amounts of this were then added to a series of glass tubes containing 10 mL F/2 medium (Guillard, 1975). The modified dilution method (Throndsen, 1978) was used for isolating a single cell. Strains were maintained in F/2 medium at a salinity of 30 g kg^{-1} at 20 °C and under a 12:12 h light:dark cycle with an approximately 810 lx illumination.

2.4. LM and SEM observation

Living cells of *K. veneficum* were observed using an Olympus CX31 (Olympus, Tokyo, Japan) and micrographs were taken using a Leica DFC 420 digital camera that was attached to a Leica DM 2500 microscope (Leica, Wetzlar, Germany). Young age cells from clonal cultures were immobilized in Lugol's solution and examined under bright field with the Leica DM 2500 microscope. Cell length and width were measured from 50 cells in mid-exponential growth phase that were photographed using a calibrated objective. The chloroplasts and nucleus were observed and photographed with a fluorescence microscope Leica DM5000B (Leica, Wetzlar, Germany).

For scanning electron microscopy (SEM) observations, the culture was first concentrated by gentle centrifugation (Bolch et al., 1999), then 0.5 mL of the condensed culture was fixed by adding an equal volume of 4% OSO4 (made up with culture medium) for 1.5 h at room temperature. The fixed cells were then rinsed once with distilled water, and dehydrated through an ethanol series (10%, 30%, 50%, 70%, 80%, 90%, 95% and 99%), allowing 10–15 min at each step. Finally two 15 min rinses each in 100% ethanol and 100% dry acetone were carried out (De Salas et al., 2008). The samples were critical-point-dried in liquid CO_2 in a Hitachi HCP-2 critical-point-drying apparatus, and subsequently glued to SEM-stubs and sputter coated with gold. Sample examination was performed using a Hitachi S-3000N scanning electron microscope.

Field samples were concentrated to 50 mL after sedimentation for more than 24 h. For observation, 1 mL subsample was transferred to a 1 mL Sedgewick Rafter counting chamber. Cells were then counted under a light microscope (Olympus CX31) at 100 × and 400 × magnification. This step was repeated if the plankton abundance was low.

2.5. Data analysis

Cell abundances (cells L^{-1}) were calculated according to the equation: cell abundance $= 100 \times C_n/V$, where C_n is the number of cells counted and V (mL) is the volume of the observed subsample.