**GROWTH INTERACTIONS BETWEEN THE BLOOM-FORMING DINOFLAGELLATES**

*Prorocentrum donghaiense* AND *Karenia mikimotoi*

**UNDER DIFFERENT TEMPERATURE**

**ABSTRACT**

Growth interactions between *Prorocentrum donghaiense* and *Karenia mikimotoi*, which are major harmful algal blooms (HABs) species in the East China Sea (ECS), were investigated by using bi-algal cultures under different temperatures. When the culture temperature was 16 °C, the growth of *P. donghaiense* and *K. mikimotoi* in bi-algal cultures had influence or no influence on each other when the cell density ratio (*P. donghaiense*: *K. mikimotoi*) was 2:1 or 1:1. *P. donghaiense* was not affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* when the cell density ratio was 1:2 and just the opposite result when the cell density ratio was 1:4. When the culture temperature was 20 °C, the growth of *K. mikimotoi* was suppressed by *P. donghaiense* in bi-algal cultures under the case that the initial density ratios were 2:1 and 1:1, two microalgae had no influence on each other when the cell density ratio was 1:2 and the growth of *P. donghaiense* was suppressed by *K. mikimotoi* when the cell density ratio was 1:4. When the culture temperature was 24 °C, the growth of *P. donghaiense* was suppressed by *K. mikimotoi* under all initial density ratios and *P. donghaiense* cannot survive in bi-algal cultures when the initial cell density ratio was 1:4; the growth of *K. mikimotoi* was not suppressed by *P. donghaiense* under the case that the initial density ratios were 1:1, 1:2 and 1:4. When the culture temperature was 28 °C, the growth of *P. donghaiense* and *K. mikimotoi* had no influence on each other when the cell density ratios were 2:1 and 1:1, *P. donghaiense* cannot survive in bi-algal cultures when the initial cell density ratios were 2:1 and 1:4. In a word, *P. donghaiense* has a survival strategy that is superior to that of *K. mikimotoi* in bi-algal cultures at low temperature (16 °C and 20 °C), *K. mikimotoi* has a survival strategy that is superior to that of *P. donghaiense* in bi-algal cultures at higher temperature (24 °C and 28 °C) and *K. mikimotoi* became dominant when the initial cell density ratio was 1:4 under four temperatures. The results showed that the culture temperature and the initial cell density play key roles in the growth interactions between *P. donghaiense* and *K. mikimotoi*.

**Key words:** Growth interaction, temperature, initial cell density, *Prorocentrum donghaiense*, *Karenia mikimotoi*.

**RESUMEN**

Se estudiaron las interacciones en el crecimiento entre *Prorocentrum donghaiense* y *Karenia mikimotoi*, que son algas componentes mayoritarios de las floraciones tóxicas (HABs) en el Mar de China del Este (ECS), mediante el uso de cultivos bialgales a diferentes temperaturas. Cuando la temperatura fue de 16 °C, hubo poca o ninguna influencia entre *P. donghaiense* y *K. mikimotoi* cuando las densidades celulares se encontraron en valores de 2:1 o 1:1 (*P. donghaiense*: *K. mikimotoi*). *P. donghaiense* no resultó afectado por *K. mikimotoi* y *K. mikimotoi* resultó afectado por *P. donghaiense* cuando la razón de densidades fue de 1:2 mientras que el resultado se invirtió cuando la razón de densidades estuvo en 1:4. Cuando el cultivo se realizó a 20 °C, se suprimió el crecimiento de *K. mikimotoi* debido a la presencia de *P. donghaiense* con razones iniciales de densidad de 2:1 y 1:1, no hubo influencia de una microalga sobre la otra cuando la razón inicial de densidad fue 1:2 y se inhibió el crecimiento de *P. donghaiense* debido al efecto de *K. mikimotoi* cuando la razón de densidad inicial fue 1:4. A 24 °C, el crecimiento de *P. donghaiense* fue suprimido por *K. mikimotoi* en todas las densidades iniciales. *P. donghaiense* no pudo sobrevivir en cultivos bialgales cuando la densidad inicial fue de 1:4. Por su parte, el crecimiento de *K. mikimotoi* no se vio afectado por *P. donghaiense* cuando las densidades relativas iniciales fueron 1:1, 1:2 y 1:4. Cuando la temperatura de los cultivos se estableció en 28 °C, no hubo influencia mutua de *P. donghaiense* y *K. mikimotoi* en valores iniciales de 2:1 y 1:1, mientras que *P. donghaiense* no sobrevivió cuando las densidades celulares iniciales fueron 1:2 y 1:4. En resumen, *P. donghaiense* posee una estrategia de supervivencia más favorable que *K. mikimotoi* en cultivos bialgales a bajas temperaturas (16 °C y 20 °C), mientras que la estrategia de supervivencia de *K. mikimotoi* es superior a la de *P. donghaiense* en las temperaturas superiores (24 °C y 28 °C). Además *K. mikimotoi* llegó a ser dominante cuando las densidades celulares iniciales fueron en la relación 1:4 en las cuatro temperaturas ensayadas. Los resultados demuestran que tanto la temperatura como las densidades relativas iniciales juegan papeles clave en las interacciones de crecimiento entre *P. donghaiense* y *K. mikimotoi*.

**Palabras clave:** Interacción de crecimiento, temperatura, densidad celular inicial, *Prorocentrum donghaiense*, *Karenia mikimotoi*. 

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INTRODUCTION

Prorocentrum donghaiense Lu (formerly named as P. dentatum, Lu & Goebel 2001; Gómez, 2005), is a dinoflagellate belonging to Prorocentrum, Procentracae, Prorocentriada. It is a major HABs species near the Yangtze Estuary and the adjacent coastal waters of the ECS during late April-May annually since 2000 (Lu & Goebel 2001; Lu et al., 2005; Tang et al., 2006). More than 120 P. donghaiense bloom events were recorded in ECS from 2001 to 2010 (Zhao, 2010), and the scale of these blooms ranged from several thousand to more than 10,000 km² and lasted from several days to one month. It is estimated that the direct loss to fisheries caused by P. donghaiense blooms is more than several million renminbi (RMB) every year (data from State Oceanic Administration in East China Sea Branch). Karenia mikimotoi Hansen (formerly named as Gymnodinium mikimotoi, Hansen et al., 2000; Gómez, 2005), is a dinoflagellate belonging to Karenia, Kareniaceae, Gymnodiniida. It is a red-tide species around the world including regions such as the eastern North Atlantic, Japan, Europe, Australia, South America, North Africa and China (reviewed by Gentien, 1998). Large scale K. mikimotoi blooms in the seas of Hong Kong in 1998 resulted in the die-off of fish, causing enormous economic losses estimated at US$ 40 million (Wang et al., 2001; Lu & Hodgkiss, 2004). K. mikimotoi blooms occurred frequently near the adjacent coastal waters of Zhejiang and Fujian province, China since 2004 (Zhao, 2010). In addition, the hemolytic and cytotoxic compounds released by K. mikimotoi have been associated with ichthyotoxicity (Neely & Campbell, 2006; Satake et al., 2005) and the toxic substances in K. mikimotoi can be transferred through marine food webs and harm humans (Chen et al., 2011).

Skeletonema costatum, P. donghaiense and K. mikimotoi are major HABs species near the Yangtze River estuary and the ECS since the 21st century, the frequency of these three HABs occurrence for nearly half of all the red tides (Zhao, 2010). Diatom blooms and dinoflagellate blooms often appears alternatively in the bloom succession (diatom-dinoflagellate-diatom). S. costatum blooms occurred in early spring when the nutrient (especially the phosphorus) were sufficient because S. costatum had a greater advantage in phosphate affinity and also had a higher phosphorus demand for growth, thus, the growth of S. costatum collapsed soon after phosphate depletion (Ou et al., 2008). P. donghaiense blooms and K. mikimotoi blooms have been observed in the coastal waters of the ECS from late April to June since 2005 (Zhou et al., 2006; Li et al., 2009; Zhao, 2010). P. donghaiense could make good use of the metabolized dissolved organic phosphorus in the water after the collapse of S. Costatum blooms to sustain their growth (Ou et al., 2008). K. mikimotoi can assimilate different kind of inorganic and phosphorus sources as well as some organic phosphorus compounds, too (Lei & Lu, 2011). S. costatum blooms became dominate after June because of the nutrients have been supplied again from the Yangtze River Diluted Water and the Taiwan Warm Current (Li et al., 2009; Zhao, 2010). It seems to be the nutrient play an important role in diatom-dinoflagellate blooms succession. However, P. donghaiense and K. mikimotoi blooms have been observed occurring in the same area from late April to June, and the nutrient in seawater are in the same level of the two species, other factors (e.g. temperature, light and so on) may play a leading role in the P. donghaiense and K. mikimotoi blooms succession.

Interactions between HABs species have been studied widely by many authors. For example, S. costatum, a typical diatom species, interactions with other species such as Gyrodinium instriatum (Nagasoe et al., 2006), P. minimum (Tameishi et al., 2009), Heterosigma akashiwo (Yamasaki et al., 2007, 2011), Chattonella marina (Wang et al., 2010) under a single temperature condition with some initial cell densities have been studied and these results showed that S. costatum has a competitive advantage compared with others. P. donghaiense which interactions with Alexandrium tamarense (Wang et al., 2006; Yang et al., 2010), Scrippsiella trochoidea (Wang & Tang, 2008), A. minutum (Yang et al., 2010) have been studied and the results indicated that P. donghaiense did not have a competitive advantage compared with others. K. mikimotoi which interactions with H. circularisquama (Uchida et al., 1999), H. akashiwo (Zhao et al., 2009), P. micans (Li et al., 2011) have been studied and the results showed that K. mikimotoi did not have a competitive advantage compared with others, either. Interspecific competition between P. donghaiense and K. mikimotoi under 25 °C with some initial cell densities has been studied and the results showed that the two microalgae had an influence on each other (Huang et al., 2009). However, most of these species form alternating blooms in various coastal waters and few two species blooms occurred together except P. donghaiense and K. mikimotoi. Therefore, it is important to realize the interactions between P. donghaiense and K. mikimotoi under different conditions.

Temperature is a key factor that affects the total abundance of phytoplankton seasonal distribution (Zhao, 2010). The phytoplankton biomass, dominant species and community structure have been changed in the Taiwan Strait and Yangtze Estuary under global Climate Changes (Hong et al., 2005; Zhang, 2009). In this case, the red tide succession will appear abnormal fluctuations, for instance, the blooms of 2005 was unusual as it begin with
K. mikimotoi blooms, followed by *P. donghaiense* blooms in Zhoushan islands area due to the higher temperature and the bloom of 2008 was *P. donghaiense* blooms which mainly occurred in Zhejiang province area because of the lower temperature (< 21°C) compared to other years (Zhao, 2010). Although both *P. donghaiense* and *K. mikimotoi* have high potential to form blooms in later-spring and early-summer near the Yangtze Estuary and the adjacent coastal waters of the ECS and even occurred together, growth interactions between two species under different temperatures in the process of blooms succession have not been carried out. In the present study, the growth interactions between *P. donghaiense* and *K. mikimotoi* by using bi-algal culture experiments under different temperatures with several combinations of initial cell densities of the two species were examined.

**RESULTS**

**Microalgal growth in bi-algal culture at 16 °C**

Fig. 1 showed the growth of *P. donghaiense* and *K. mikimotoi* in bi-algal cultures with different initial cell densities at 16 °C. When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.4 × 10^4 cells ml^-1 and 0.2 × 10^4 cells ml^-1, respectively (Fig. 1A), the growth of *P. donghaiense* in bi-algal culture was almost identical as in the mono-algal culture during the treatment time of 11 d and significantly suppressed at 12d (*P* = 0.0201 < 0.05). The results showed the two microalgae had influence on each other under this cell density ratio (2:1). When the initial cell densities of both *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 (Fig. 1B), the growth of *P. donghaiense* in bi-algal culture was almost identical as in the mono-algal culture during the treatment of 11 d, and weakly suppressed at 12d (*P* = 0.1105 > 0.05). The growth of *K. mikimotoi* in bi-algal culture was almost identical as in the mono-algal culture during the treatment time of 11 d, and the cell densities decreased at 12d (*P* = 0.2873 > 0.05). The growth of *K. mikimotoi* in bi-algal culture and in the mono-algal culture were alike during the treatment time of 11 d, and the cell densities decreased at 12d (*P* = 0.0052 < 0.05). The results showed *P. donghaiense* was not affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* under this cell density ratio (1:2). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 and 0.8 × 10^4 cells ml^-1, respectively (Fig. 1C), the growth of *P. donghaiense* in bi-algal culture was almost identical as in the mono-algal culture during the treatment time of 11 d and significantly suppressed at 12d (*P* = 0.0074 < 0.05). The growth of *K. mikimotoi* in bi-algal culture was almost identical as in the mono-algal culture during the treatment time of 11 d and significantly suppressed at 12d (*P* = 0.0201 < 0.05). The results showed the two microalgae had influence on each other under this cell density ratio (2:1). When the initial cell densities of both *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 (Fig. 1B), the growth of *P. donghaiense* in bi-algal culture was almost identical as in the mono-algal culture during the treatment of 11 d, and weakly suppressed at 12d (*P* = 0.0775 > 0.05), too. The results showed the two microalgae had no influence on each other under this cell density ratio (1:1). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 and 0.4 × 10^4 cells ml^-1, respectively (Fig. 1C), the growth of *P. donghaiense* in bi-algal culture and in the mono-algal culture were alike during the treatment time of 11 d, and the cell densities decreased at 12d (*P* = 0.2873 > 0.05). The growth of *K. mikimotoi* in bi-algal culture and in the mono-algal culture were alike during the treatment time of 11 d, and the cell densities decreased at 12d (*P* = 0.0052 < 0.05). The results showed *P. donghaiense* was not affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* under this cell density ratio (1:2). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 and 0.8 × 10^4 cells ml^-1, respectively (Fig. 1D), the growth of *P. donghaiense*
in bi-algal culture was almost identical as in the mono-algal culture during the treatment time of 11 d, but the growth of *P. donghaiense* was significantly suppressed at 12d (*P* = 0.0197 < 0.05), the cell density of *P. donghaiense* was about 55% of that in mono-algal culture. The growth of *K. mikimotoi* in bi-algal culture was almost the same as in the mono-algal culture during 12 d and the cell density was a little less than the mono-algal culture (*P* = 0.0721 > 0.05). The results showed *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P. donghaiense* under this cell density ratio (1:4).

**Microalgal growth in bi-algal culture at 20 °C**

Fig. 2 showed the growth of *P. donghaiense* and *K. mikimotoi* in bi-algal cultures with different initial cell densities at 20 °C. When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.4 × 10^4 cells ml^-1 and 0.2 × 10^4 cells ml^-1, respectively (Fig. 2A), the growth of *P. donghaiense* in bi-algal culture was almost the same as in the mono-algal culture during the treatment time of 11 d, and the growth of *P. donghaiense* was weakly suppressed at 12d (*P* = 0.2726 > 0.05) and the cell density of *P. donghaiense* was notably higher than the cell density *K. mikimotoi* from 6d to 11d. The growth of *K. mikimotoi* in bi-algal culture and in the mono-algal culture were alike during the treatment time of 11 d, and the growth of *K. mikimotoi* was suppressed at 12d (*P* = 0.0049 < 0.05). The results showed *P. donghaiense* was not affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* under this cell density ratio (2:1). When the initial cell densities of both *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 (Fig. 2B), the growth of *P. donghaiense* in bi-algal culture was almost the same as in the mono-algal culture during the treatment time of 9 d, and the cell density decreased at 12d (*P* = 0.0791 > 0.05). The growth of *K. mikimotoi* in bi-algal culture was almost the same as in the mono-algal culture during 11d and suppressed at 12d (*P* = 0.0086 < 0.05). The results showed *P. donghaiense* was not affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* under this cell density ratio (1:1). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 and 0.4 × 10^4 cells ml^-1, respectively (Fig. 2C), the growth of *P. donghaiense* in bi-algal culture was alike to that in the mono-algal culture during 12d (*P* = 0.0583 > 0.05). The growth of *K. mikimotoi* in bi-algal culture was alike to that in the mono-algal culture during 12d (12d: *P* = 0.4763 > 0.05), too. The results showed *P. donghaiense* and *K. mikimotoi* had no influence on each other in bi-algal culture under this cell density ratio (1:2). When initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^-1 and 0.8 × 10^4 cells ml^-1, respectively (Fig. 2D), the growth of *P. donghaiense* in bi-algal culture was significantly suppressed from 7d to 12d (*P* = 0.0121, 0.0148, 0.0016, 0.0116, 0.0289, 0.0248, respectively). The growth of *K. mikimotoi* in bi-algal culture was better than in the mono-algal culture during 12 d. The results showed *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P.
**Microalgal growth in bi-algal culture at 24 °C**

Fig. 3 showed the growth of *Prorocentrum donghaiense* and *Karenia mikimotoi* in bi-algal cultures with different initial cell densities at 24 °C. When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.4 × 10^4 cells ml^(-1) and 0.2 × 10^4 cells ml^(-1), respectively (Fig. 3A), the growth of *P. donghaiense* was better than control maybe by the stimulation of *P. donghaiense* under this cell density ratio (1:4), and even the growth of *K. mikimotoi* was better than control mostly by the stimulation of *P. donghaiense*.

*Microalgal growth in bi-algal culture at 24 °C*

When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.4 × 10^4 cells ml^(-1) and 0.2 × 10^4 cells ml^(-1), respectively (Fig. 3A), the growth of *P. donghaiense* was better than control in bi-algal culture at 24 °C. When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^(-1) and 0.8 × 10^4 cells ml^(-1), respectively (Fig. 3C), the growth of *P. donghaiense* in bi-algal culture was almost the same as in the mono-algal culture during 12d (12d: *P* = 0.0534 > 0.05). The results showed that *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was affected by *P. donghaiense* under this cell density ratio (1:2). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^(-1) and 0.8 × 10^4 cells ml^(-1), respectively (Fig. 3D), the growth of *P. donghaiense* in bi-algal culture was suppressed from 9-12d (*P* = 0.0036, 0.0095, 0.0116, 0.0090, respectively). The growth of *K. mikimotoi* in bi-algal culture was almost the same as in the mono-algal culture during 12d (12d: *P* = 0.0534 > 0.05). The results showed that *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P. donghaiense* under this cell density ratio (1:1). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^(-1) and 0.4 × 10^4 cells ml^(-1), respectively (Fig. 3B), the growth of *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P. donghaiense* under this cell density ratio (1:2). When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.2 × 10^4 cells ml^(-1) and 0.4 × 10^4 cells ml^(-1), respectively (Fig. 3D), the growth of *P. donghaiense* in bi-algal culture was suppressed from 9-12d (*P* = 0.0096 and 0.0062) and all *P. donghaiense* were killed at 10d. The growth of *K. mikimotoi* in bi-algal culture was almost identical as in the mono-algal culture during 12d (12d: *P* = 0.4075 > 0.05). The results showed that *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P. donghaiense* under this cell density ratio (1:4).

**Microalgal growth in bi-algal culture at 28°C**

Fig. 4 showed the growth of *Prorocentrum donghaiense* and *Karenia mikimotoi* in bi-algal cultures with different initial cell densities at 28 °C. When the initial cell densities of *P. donghaiense* and *K. mikimotoi* were 0.4 × 10^4 cells ml^(-1) and 0.2 × 10^4 cells ml^(-1), respectively (Fig. 4A), the growth of *P. donghaiense* was affected by *K. mikimotoi* and *K. mikimotoi* was not affected by *P. donghaiense* under this cell density ratio (1:4).
Growth of *P. donghaiense* and *K. mikimotoi* in the bi-algal culture with four initial cell density ratios at 28°C: (A) 2:1; (B) 1:1; (C) 1:2; (D) 1:4. The initial cell density of *P. donghaiense* in the bi-algal culture were set at: 0.2 × 10^4 cells ml^-1 (P1) and 0.4 × 10^4 cells ml^-1 (P2), and the initial cell density of *K. mikimotoi* in the bi-algal culture were set at: 0.8 × 10^4 cells ml^-1 (K4); 0.4 × 10^4 cells ml^-1 (K2) and 0.2 × 10^4 cells ml^-1 (K1). ● Growth of *P. donghaiense* in control; ○ Growth of *P. donghaiense* in the bi-algal culture; ◯ Growth of *K. mikimotoi* in the bi-algal culture; □ Growth of *K. mikimotoi* in control; △ Growth of *K. mikimotoi* in the mono-algal culture.

Figure 4:

Role of temperature in competition between *P. donghaiense* and *K. mikimotoi*

Nutrients, temperature, salinity, light and so on are important environmental factors related to microalgae growth, and affect the population dynamics of individual species and species succession in the field (Karentz & Smayda, 1984). In the field, *P. donghaiense* blooms have been observed in May near the adjacent coastal waters of the north area such as Zhoushan Islands and *K. mikimotoi* blooms have been observed in June near the adjacent coastal waters of the south area such as Dongtou Islands (Zhao, 2010). The optimal temperature for growth of *P. donghaiense* was 18-21°C (Wang, 2003) and long-term monitoring data of Zhoushan Islands showed that *P. donghaiense* blooms became dominate when the surface sea temperature (SST) was below 20°C and the population of *P. donghaiense* collapsed rapidly when the SST was
above 23 °C (Wang & Huang, 2003; Zhao, 2010). The optimal temperature for the growth of *K. mikimotoi* was 23 °C (Yao et al., 2007). In this study, *P. donghaiense* has an advantage in the competition with *K. mikimotoi* when the sea water temperature is low (16-20°C). *K. mikimotoi* has an advantage in the competition with *P. donghaiense* when the sea water temperature is high (24-28°C). This result is very similar in the rules of *P. donghaiense* or *K. mikimotoi* blooms occurrence, which showed that 80% PD-blooms occurred blow 22 °C and 74% KM-blooms occurred above 22 °C in the ECS from 2001 to 2010 (Unpublished data). Therefore, Temperature plays a very important role in the process of dinoflagellate blooms succession. However, not much is known about how changes in temperature affect phytoplankton allelopathy. For example, the haemolytic activity of *Phaeocystis pouchetii* at 4 °C was approximately half the activity measured at 15 °C (van Rijssel et al., 2007). In addition, there was no difference between the effects of filtrate obtained from *A. tamarense* at 14 °C and 20 °C on *S. trochoidea* or *Heterocapsa trquetra* (Fistarel et al., 2004). Wang & Tang, 2008 reported that the pre-treated (maintained water bath at 30, 60 and 100 °C, for 30mins) *S. trochoidea* filtrate inhibited *P. donghaiense* growth during the first 120 h and then significantly increased its growth and ordered by 100 °C > 60 °C > 30 °C > control by the end, but the same pre-treated *P. donghaiense* filtrate inhibited *S. trochoidea* growth from 72 h till the end of the experiment. There are different allelopathic substance(s) of different harmful algal species (Granéli et al., 2008) and have different thermal stability. In general, allelopathic substance(s) have been degraded easily in high temperature environment (higher than that of the suitable algae growth temperature) which will diminish the toxic effects on the target organisms, on the other hand, more allelopathic substance(s) will be released by algae in higher temperature environment (during suitable algae growth temperature). Further study is needed to determine the effect of the culture filtrate of *K. mikimotoi* and *P. donghaiense* on each other.

### Role of initial cell density in competition between *P. donghaiense* and *K. mikimotoi*

Allelopathy and cell contact are important factors in the competition between phytoplankton species (Granéli & Johansson, 2003; Granéli & Hansen, 2006; Uchida et al., 1995, 1999; Uchida, 2001). The allelopathic effect and lethal effect by cell contact which depends on the cell density of the donor and the target species (Uchida et al., 1999; Schmidt & Hansen, 2001). Uchida et al. (1999) had reported that *H. circularisquama* would become a temporary cyst after 59 contacts with *K. mikimotoi* and *P. donghaiense* would be killed after 19 contacts with *H. circularisquama*. Thus, the initial cell densities of both toxic algae and its target seem to be important for the outcome of the interaction (Granéli & Hansen, 2006). For instance, when the initial cell density of *P. donghaiense* was low (1.0 × 10^5 cells ml^-1), the growth of *P. donghaiense* was dramatically suppressed and finally

### Table 1:

Estimated parameters from equations in mono-algal and bi-algal cultures of *P. donghaiense* (PD) and *K. mikimotoi* (KM) with different temperature and initial cell density ratio at 16 °C.

<table>
<thead>
<tr>
<th>Initial cell density ratio (PD:KM)</th>
<th>Carrying capacity, K (Cells ml^-1)</th>
<th>Growth rate, r (Divisions day^-1)</th>
<th>Interaction rate A or B (ml cell^-1 s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1 PD</td>
<td>168,777</td>
<td>0.380</td>
<td>5.166 1.16 × 10^5</td>
</tr>
<tr>
<td>KM</td>
<td>71,190,036</td>
<td>0.437</td>
<td>5035.376 3.09 × 10^5</td>
</tr>
<tr>
<td>1:1 PD</td>
<td>37,312,465</td>
<td>0.335</td>
<td>2469.654 2.22 × 10^5</td>
</tr>
<tr>
<td>KM</td>
<td>71,190,036</td>
<td>0.437</td>
<td>5255.911 3.23 × 10^5</td>
</tr>
<tr>
<td>1:2 PD</td>
<td>37,312,465</td>
<td>0.335</td>
<td>1152.001 1.03 × 10^5</td>
</tr>
<tr>
<td>KM</td>
<td>208,201,053</td>
<td>0.477</td>
<td>16099.570 3.69 × 10^5</td>
</tr>
<tr>
<td>1:4 PD</td>
<td>37,312,465</td>
<td>0.335</td>
<td>1265.701 1.14 × 10^5</td>
</tr>
<tr>
<td>KM</td>
<td>422,396</td>
<td>0.415</td>
<td>27.089 2.66 × 10^5</td>
</tr>
</tbody>
</table>
all *P. donghaiense* cells were killed by *A. tamarense* (the initial cell density was $2.8 \times 10^4$ cells ml$^{-1}$) in bi-algal cultures, and when the initial cell density of *P. donghaiense* was high ($1.0 \times 10^5$ cells ml$^{-1}$) and the initial cell density of *A. tamarense* was unchanged, the growth of *P. donghaiense* was significantly suppressed compared to the control, but no outcompetetment was observed in the end (Wang et al., 2006). *K. mikimotoi* became dominant when the initial cell density ratios of *H. akashiwo* and *K. mikimotoi* were 1:1 and 1:4, and *H. akashiwo* overcame *K. mikimotoi* when the initial cell density ratios of *H. akashiwo* and *K. mikimotoi* was 4:1 (Zhao et al., 2009). When the initial cell densities of *S. castatum* and *H. akashiwo* were $10^2$ cells ml$^{-1}$ and $10^4$ cells ml$^{-1}$, respectively, the growth of *H. akashiwo* was virtually the same as in both bi-algal and mono-algal cultures, and the growth of *S. castatum* was remarkably suppressed in bi-algal cultures, when the initial cell densities of *S. castatum* and *H. akashiwo* were $10^2$ cells ml$^{-1}$ and $10^5$ cells ml$^{-1}$, the growth of the two species were completely reversed (Yamasaki et al., 2007). The similar results were observed in other species competitions such as *H. akashiwo* and *P. minimum* (Yamasaki et al., 2010), *H. akashiwo* and *Chattonella antiqua* (Qiu et al., 2011). In the present study, *K. mikimotoi* became dominant when the initial cell density ratio of *P. donghaiense* and *K. mikimotoi* was 1:4 at 16, 20, 24 and 28°C, respectively, and the higher the temperature, the more obvious competitive advantage (Fig. 1, 2, 3, and 4). *P. donghaiense* became dominant when the initial cell density ratio of *P. donghaiense* and *K. mikimotoi* was 2:1 at 16 and 20°C, and the higher the temperature, the less obvious competitive advantage (Fig. 1, 2, 3, and 4). Thus, it serves as an important role of the initial cell density in competition between *P. donghaiense* and *K. mikimotoi*.

### Growth simulation in bi-algal cultures

The growth of *P. donghaiense* and *K. mikimotoi* in bi-algal cultures with different temperature and initial cell density ratio was simulated using the following equations (Uchida et al., 1999):

$$
\frac{dx}{dt} = r_x \left[ \left( 1 - x \right) K_x^{-1} \right] - A_x = r_x \left[ 1 - (x + ay) K_x^{-1} \right] \quad (1)
$$

$$
\frac{dy}{dt} = r_y \left[ \left( 1 - y \right) K_y^{-1} \right] - B_y = r_y \left[ 1 - (bx + y) K_y^{-1} \right] \quad (2)
$$

Here, $x$ and $y$ are the cell densities of *P. donghaiense* and *K. mikimotoi*, respectively. The $r_x$ and $K_x$ are the growth rate and carrying capacity of *P. donghaiense*, and $r_y$ and $K_y$ are the growth rate and carrying capacity of *K. mikimotoi* when each species is cultured in a mono-algal culture. $A$ measures the degree of inhibition of *P. donghaiense* by *K. mikimotoi*, and $B$ vice versa, the parameters $A$ and $B$ are calculated from Eqs. $A = ar_x K_x^{-1}$ and $B = br_y K_y^{-1}$, respectively. Parameters $a$ and $b$ are non-dimensional, indicating the degree of inhibition by the other species when compared to its self-interference.

Eqs. (1) and (2) can be approximated with the following equations:

### Table 2:

Estimated parameters from equations in mono-algal and bi-algal cultures of *P. donghaiense* (PD) and *K. mikimotoi* (KM) with different temperature and initial cell density ratio at 20°C.

<table>
<thead>
<tr>
<th>Initial cell density ratio (PD:KM)</th>
<th>Carrying capacity, $K$ (Cells ml$^{-1}$)</th>
<th>Growth rate, $r$ (Divisions day$^{-1}$)</th>
<th>Interaction rate $A$ or $B$ (ml cell$^{-1}$ day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1 PD</td>
<td>68,471</td>
<td>0.661</td>
<td>0.520 $5.02 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>33,968,107</td>
<td>0.464</td>
<td>496.275 $6.78 \times 10^6$</td>
</tr>
<tr>
<td>1:1 PD</td>
<td>53,083</td>
<td>0.700</td>
<td>0.408 $5.38 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>33,968,107</td>
<td>0.464</td>
<td>368.615 $5.04 \times 10^6$</td>
</tr>
<tr>
<td>1:2 PD</td>
<td>53,083</td>
<td>0.700</td>
<td>0.744 $9.81 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>287,232</td>
<td>0.492</td>
<td>7.165 $1.23 \times 10^5$</td>
</tr>
<tr>
<td>1:4 PD</td>
<td>53,083</td>
<td>0.700</td>
<td>0.431 $5.69 \times 10^6$</td>
</tr>
<tr>
<td></td>
<td>227,434</td>
<td>0.718</td>
<td>3.783 $1.19 \times 10^5$</td>
</tr>
</tbody>
</table>
Table 3:
Estimated parameters from equations in mono-algal and bi-algal cultures of *P. donghaiense* (PD) and *K. mikimotoi* (KM) with different temperature and initial cell density ratio at 24 °C.

<table>
<thead>
<tr>
<th>Initial cell density ratio (PD:KM)</th>
<th>Carrying capacity, ( K ) (Cells ml(^{-1}))</th>
<th>Growth rate, ( r ) (Divisions day(^{-1}))</th>
<th>Interaction rate ( A ) or ( B ) (ml cell(^{-1}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>PD 36,366,180</td>
<td>0.255</td>
<td>1158.081</td>
</tr>
<tr>
<td></td>
<td>KM 46,106,434</td>
<td>0.512</td>
<td>779.840</td>
</tr>
<tr>
<td>1:1</td>
<td>PD 111,739</td>
<td>0.378</td>
<td>5.849</td>
</tr>
<tr>
<td></td>
<td>KM 46,106,434</td>
<td>0.512</td>
<td>1828.736</td>
</tr>
<tr>
<td>1:2</td>
<td>PD 111,739</td>
<td>0.378</td>
<td>0.620</td>
</tr>
<tr>
<td></td>
<td>KM 2,402,741</td>
<td>0.411</td>
<td>52.796</td>
</tr>
<tr>
<td>1:4</td>
<td>PD 111,739</td>
<td>0.378</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>KM 410,206</td>
<td>0.575</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: “--” out-competition was observed in bi-algal culture and the interaction rate cannot be estimated.

\[
\begin{align*}
(Ln x_{t_i} - Ln x_{t_0}) (t_{t_i} - t_{t_0})^{-1} &= r_x - r_x K_x - r_o y K_o \quad (3) \\
(Ln y_{t_i} - Ln y_{t_0}) (t_{t_i} - t_{t_0})^{-1} &= r_y - r_y K_y - r_o x K_o \quad (4)
\end{align*}
\]

Here, \( x \) and \( y \) denote the observed cell densities at time \( t \) for *P. donghaiense* and *K. mikimotoi*, respectively. When each species is cultured in a mono-algal culture, \( a = b = 0 \) can be set. The logistic parameters are estimated by Eqs. (3) and (4) using the mono-algal culture data. The parameters \( a \) and \( b \) are calculated using bi-algal culture data directly from Eqs. (3) and (4).

The values of all parameters used in the growth simulations are shown in Table 1, 2, 3 and 4. The carrying capacities for *P. donghaiense* \( (K) \) were lower than the carrying capacity for *K. mikimotoi* \( (K) \) except some treatments at 16 °C. The growth rates of *P. donghaiense* \( (r_x) \) were higher at 20 °C \( (r_x = 0.661 \text{ and } 0.700 \text{ divisions day}^{-1}) \) than others temperature groups \( (r_x = 0.255 \text{ to } 0.489 \text{ divisions day}^{-1}) \), and the growth rates of *K. mikimotoi* \( (r_y) \) were higher than *P. donghaiense* \( (r_x) \) except some treatments at 20 °C, the values of \( r_y \) were estimated approximately from 0.411 to 0.806 divisions day\(^{-1}\). The parameters \( A \) and \( B \) were very close in most of the treatment groups and the ratio of \( A \) and \( B \) were mostly around 1 (Table 1, 2, 3 and 4), for example, the value of \( A \) for *P. donghaiense* was \( 5.38 \times 10^{4} \text{ ml cell}^{-1} \text{ s}^{-1} \) and \( B \) for *K. mikimotoi* was \( 5.04 \times 10^{4} \text{ ml cell}^{-1} \text{ s}^{-1} \) when the initial cell density ratio was 1:1 at 20 °C (Table 2). The model simulation that was based on the results of these bi-algal culture experiments predicted that *P. donghaiense* and *K. mikimotoi* will co-exist in bi-algal cultures under most experimental conditions. Several previous studies have used this model to examine the growth interactions between *P. donghaiense* or *K. mikimotoi* and other species. Uchida et al. (1999) reported that the degree of inhibition on *K. mikimotoi* by *H. circularisquama* was three times larger than the degree of inhibition on *H. circularisquama* by *K. mikimotoi* and indicated that *K. mikimotoi* was killed by cell contact with *H. circularisquama* and *H. circularisquama* was suppressed by *K. mikimotoi* because of allelopathic substances. Wang et al. (2006) reported that the inhibitory strength of *A. tamarense* on *P. donghaiense* was 17 times higher than the inhibitory strength of *P. donghaiense* on *A. tamarense* when the initial cell density ratio was 3.6:1 and the inhibitory strength of *A. tamarense* on *P. donghaiense* was 8 times higher than the inhibitory strength of *P. donghaiense* on *A. tamarense* when the initial cell density ratio was 36:1. The growth interactions between *P. donghaiense* and *S. trochoidea* was reported and the results showed that the inhibitory strength of *S. trochoidea* on *P. donghaiense* were 43 and 24 times higher than the inhibitory strength of *P. donghaiense* on *S. trochoidea* when the initial cell density ratio were 1.9:1 and 19:1, respectively (Wang & Tang, 2008). Therefore, the growth interactions between two different species appear to be interspecies-specific and the mechanisms behind those interactions (allelopathy or cell contact and even two of them) can vary from species to species (Tameishi et al., 2009). However, in the present study,
The growth patterns of the two species as predicted using the model (Uchida et al., 1999) are not necessarily similar to those analyzed based on student’s t-test. For instance, when *P. donghaiense* and *K. mikimotoi* in bi-algal culture at 20 °C, *P. donghaiense* was not affected by *K. mikimotoi* in bi-algal culture when the cell density ratios were 2:1, 1:1 and 1:2, respectively (Fig. 2), when *P. donghaiense* and *K. mikimotoi* in bi-algal culture at 24 °C, *P. donghaiense* were affected by *K. mikimotoi* in bi-algal culture in all cell density ratios (Fig. 3). *P. donghaiense* has a survival strategy superior to that of *K. mikimotoi* when the temperature was 20 °C and *K. mikimotoi* has a survival strategy superior to that of *P. donghaiense* when the temperature was 24 °C. The causes of this result may have the following two aspects. On the one hand, the parameters, which can be calculated, were used to model predictions are based on results obtained under fixed environmental conditions of short-term (12d) at which time the two species can coexist. On the other hand, the carrying capacity for *P. donghaiense* (*Kx*) or *K. mikimotoi* (*Ky*) was considerably larger than the cell density of *P. donghaiense* or *K. mikimotoi* in bi-algal cultures, which indicated that the two species can survive normally under this environmental condition. Further study is necessary to determine whether the degree of inhibition between *P. donghaiense* and *K. mikimotoi* under long-term (>12d). Furthermore, it is necessary to better integrate parameters for interactions between phytoplankton species into existing models for more accurate prediction of interspecific competition.

In conclusions, results of the present study demonstrated that growth interactions between *P. donghaiense* and *K. mikimotoi* under different temperatures indicated that *P. donghaiense* has an advantage at low temperature and *K. mikimotoi* has an absolutely advantage at high temperature and out-competements were observed in bi-algal cultures by the end when the initial cell density ratio of *P. donghaiense* and *K. mikimotoi* was 1:2 or 1:4 at 24°C and 28°C. In addition, it serves as an important role in the initial cell density in competition between *P. donghaiense* and *K. mikimotoi*. In fact, the phosphorus concentration in the adjacent coastal waters of the ECS was low when *P. donghaiense* blooms or *K. mikimotoi* blooms occurred (Zhao, 2010), thus, the growth interactions between *P. donghaiense* and *K. mikimotoi* under different temperatures in phosphorus limitation condition will be determined. Allelopathy of harmful dinoflagellate may play a key role in the growth dynamics of blooms and the effects of the culture filtrate of *P. donghaiense* and *K. mikimotoi* on each species under different environmental conditions will be conducted, too.

**MATERIALS AND METHODS**

**Seawater preparation**

Seawater was collected from the East China Sea near Shengshan Islands, Zhoushan city, Zhejiang province (30°45′N, 122°50′E), whose pH and salinity were 8.0 and 28, respectively. This seawater was used in the cultures for all experiments of the present study.

---

<table>
<thead>
<tr>
<th>Initial cell density ratio (PD:KM) Carrying capacity, $K$ (Cells ml$^{-1}$)</th>
<th>Growth rate, $r$ (Divisions day$^{-1}$)</th>
<th>Interaction rate $A$ or $B$ (ml cell$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>189,667</td>
<td>0.300</td>
</tr>
<tr>
<td>KM</td>
<td>216,068</td>
<td>0.680</td>
</tr>
<tr>
<td>PD</td>
<td>54,681</td>
<td>0.489</td>
</tr>
<tr>
<td>KM</td>
<td>216,068</td>
<td>0.680</td>
</tr>
<tr>
<td>PD</td>
<td>54,681</td>
<td>0.489</td>
</tr>
<tr>
<td>KM</td>
<td>243,240</td>
<td>0.629</td>
</tr>
<tr>
<td>PD</td>
<td>54,681</td>
<td>0.489</td>
</tr>
<tr>
<td>KM</td>
<td>218,329</td>
<td>0.806</td>
</tr>
</tbody>
</table>

Note: “--” out-competement was observed in bi-algal culture and the interaction rate cannot be estimated.
**Algal species and culture conditions**

*P. donghaiense* was provided by Prof. Lu of the second institute of oceanography, State Oceanic Administration, in Hangzhou, China. *K. mikimotoi* was obtained from East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai, China. They were grown in modified f/2 medium (Guillard, 1975), which was based on autoclaved (121 °C, 20 min) seawater, at 20°C and a light intensity of 65-70 µmol m⁻²s⁻¹ under a 12:12 h light: dark cycle in illuminating incubators (MTI-201B, RIKAKIKAI, JAPAN). All cultures were shaken manually twice daily at a set time. The microalgae were cultivated to the exponential growth phase for use. During the experiment, cultures were maintained in 100 ml Erlenmeyer flasks containing 50 ml fresh f/2 enriched seawater. These microalgal cultures were under the different temperatures (16°C, 20°C, 24°C and 28°C) and different initial cell densities (0.2 × 10⁴ cells ml⁻¹, 0.4 × 10⁴ cells ml⁻¹ and 0.8 × 10⁴ cells ml⁻¹), which called “monocultures”, and were used as controls throughout the experiment. A 0.5 ml sample was collected and preserved in Lugol’s solution to monitor the growth of the microalgae by direct counting the cell numbers using a haemocytometer under an optical microscope (BX43, OLYMPUS, JAPAN) at 0, 4, 6, 7, 8, 9, 10, 11 and 12 d, respectively.

**Growth interactions between P. donghaiense and K. mikimotoi in bi-algal cultures**

Bi-algal culture experiments were conducted in 100 ml Erlenmeyer flask containing 50 ml of *P. donghaiense* cells in stationary phase (stock culture: 1.0 × 10⁵ cells ml⁻¹) and were inoculated to a final cell density of 0.2 × 10⁴ cells ml⁻¹ or 0.4 × 10⁴ cells ml⁻¹ in all combinations into cultures of *K. mikimotoi* (stock culture: 1.0 × 10⁵ cells ml⁻¹) with cell densities of 0.2 × 10⁴ cells ml⁻¹ or 0.4 × 10⁴ cells ml⁻¹ or 0.8 × 10⁴ cells ml⁻¹ at 16°C, 20°C, 24°C and 28°C, respectively. The resulting combinations of initial cell densities of *P. donghaiense* and *K. mikimotoi* were, respectively, (1) 0.4 × 10⁴ cells ml⁻¹ and 0.2 × 10⁴ cells ml⁻¹; (2) 0.2 × 10⁴ cells ml⁻¹ each; (3) 0.2 × 10⁴ cells ml⁻¹ and 0.4 × 10⁴ cells ml⁻¹; (4) 0.2 × 10⁴ cells ml⁻¹ and 0.8 × 10⁴ cells ml⁻¹. Three replicate Erlenmeyer flasks were used for each treatment. All Erlenmeyer flasks were shaken manually twice daily at a set time and randomly rearranged to minimize the effects of light or temperature gradients in the incubator. A 0.5 ml sample was collected and preserved in Lugol’s solution to monitor the growth of the microalgae by direct counting the cell numbers using a haemocytometer under an optical microscope (BX43, OLYMPUS, JAPAN) at 0, 4, 6, 7, 8, 9, 10, 11 and 12 d, respectively. When cell densities exceeded 5 × 10⁴ cells ml⁻¹, subsamples were diluted 4 × to 10 × with fresh f/2 medium before counting.

**STATISTICAL ANALYSES**

Data were presented as mean ± SD of three replicates. Student’s t-test was used to examine the difference in growth between experimental groups and the control (*P* < 0.05: significant or *P* < 0.01: extremely significant). All analyses were conducted using Excel 2010 and PASW Statistics 18.0.

**ACKNOWLEDGEMENTS**

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