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Effects of temperature, salinity, and irradiance on the growth of harmful algal bloom species *Phaeocystis globosa* Scherffel (Prymnesiophyceae) isolated from the South China Sea*

XU Ning (徐宁)[1, **], HUANG Bozhu (黄博珠)[1], HU Zhangxi (胡章喜)[2], TANG Yingzhong (唐赢中)[2], DUAN Shunshan (段舜山)[1], ZHANG Chengwu (张成武)[1]

[1] *Institute of Hydrobiology*, *Jinan University*, *Guangzhou 510632*, *China*

[2] *Key Laboratory of Ecology and Environmental Sciences*, *Institute of Oceanology*, *Chinese Academy of Sciences*, *Qingdao 266071*, *China*

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Abstract Blooms of *Phaeocystis globosa* have been frequently reported in Chinese coastal waters, causing serious damage to marine ecosystems. To better understand the ecological characteristics of *P. globosa* in Chinese coastal waters that facilitate its rapid expansion, the effects of temperature, salinity and irradiance on the growth of *P. globosa* from the South China Sea were examined in the laboratory. The saturating irradiance for the growth of *P. globosa* (I_s) was 60 μmol/(m^2·s), which was lower than those of other harmful algal species (70–114 μmol/(m^2·s)). A moderate growth rate of 0.22/d was observed at 2 μmol/(m^2·s) (the minimum irradiance in the experiment), and photo-inhibition did not occur at 230 μmol/(m^2·s) (the maximum irradiance in the experiment). Exposed to 42 different combinations of temperatures (10–31°C) and salinities (10–40) under saturating irradiance, *P. globosa* exhibited its maximum specific growth rate of 0.80/d at the combinations of 24°C and 35, and 27°C and 40. The optimum growth rates (>0.80/d) were observed at temperatures ranging from 24 to 27°C and salinities from 35 to 40. While *P. globosa* was able to grow well at temperatures from 20°C to 31°C and salinities from 20 to 40, it could not grow at temperatures lower than 15°C or salinities lower than 15. Factorial analysis revealed that temperature and salinity has similar influences on the growth of this species. This strain of *P. globosa* not only prefers higher temperatures and higher salinity, but also possesses a flexible nutrient competing strategy, adapted to lower irradiance. Therefore, the *P. globosa* population from South China Sea should belong to a new ecotype. There is also a potentially high risk of blooms developing in this area throughout the year.

Keyword: *Phaeocystis globosa*; harmful algal bloom; temperature; salinity; irradiance; growth

1 INTRODUCTION

The first record of a *Phaeocystis* bloom in China was from Zhelin Bay, South China Sea in the fall of 1997, covering over 3 000 km^2 and lasting for about 6 months (Chen et al., 1999). The bloom resulted in massive fish mortalities, and severely devastated the net-cage fish aquaculture in the Fujian and Guangdong Provinces. It was estimated that the direct economic loss was more than 180 million RMB (about 26 million US dollars; Wang et al., 2007). A further study confirmed that the causative species could produce hemolytic toxins, which were probably responsible for the massive fish kills (He et al., 1999). The causative organism was identified as *Phaeocystis globosa* rather than *Phaeocystis pouchetii* or other species, based on microscopic morphological observations, culturing behavior, physiological characteristics and sequence analysis of its partial 18S rDNA (Shen et al., 2000; Wang et al., 2000; Chen et al., 2002; Xu et al., 2003; Qi et al., 2004). During the following decade, *P. globosa* blooms were

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** Corresponding author: txuning@163.com

frequently reported in Chinese coastal waters throughout the year, involving extensive areas from the Bohai Sea (the North China Sea) to the coast of the Hainan Province (the South China Sea), causing heavy damage to local fisheries (Wang et al., 2007). Owing to its frequent occurrence, broad spread and severe damage to fisheries, tourism and marine ecosystems in the past decade, *P. globosa* is thought to be one of the most important harmful algal bloom causative species in China (Qi et al., 2004; Wang et al., 2007; Liu et al., 2010).

In addition to temperate and sub-tropical coastal waters like the southeastern coastal waters of China, *Phaeocystis* blooms have also been frequently recorded in Polar Regions, the North Sea and the Arabian Sea (Garrison et al., 1987; Lancelot et al., 1987; Al-Hasan et al., 1990; Schoemann et al., 2005). It is considered to be an ecosystem disruptive algal bloom (EDAB) species (Sunda et al., 2006), endangering marine ecosystems and being hazardous to human beings. Furthermore, *Phaeocystis* is also a major producer of sulphur compounds (dimethyl sulphide) and thus is treated as a special phytoplankton group in Ocean Biogeochemical Climate Models (OBCM), which address the role of marine phytoplankton and the 'biological pump' in the oceanic biogeochemical cycles (Schoemann et al., 2005). As well as the different *Phaeocystis* species, there is also increasing evidence that the most widespread species, *P. globosa*, can be subdivided into at least five different ecotypes, which differ in their ecophysiological properties (Riegman and van Boekel, 1996). Most research has been performed on the *P. globosa* ecotype North European (English Channel/North Sea isolates) (Riegman and van Boekel, 1996). It is noteworthy that the ecological features of *P. globosa* populations occurring in the South China Sea are quite different from those in other geographic areas. For example, it was reported that water temperature reached 30°C during a *P. globosa* bloom in coastal regions of the Guangdong Province in July 1999 (Xu et al., 2003), while suitable temperatures for other strains are below 16°C (Jahnke and Baumann, 1987; Riegman and van Boekel, 1996). Furthermore, colony diameter in the Guangdong Province was up to 30 mm, much greater than previously reported (8–9 mm) (Jahnke and Baumann, 1987).

Harmful algal blooms (HABs) are often linked to nutrient enrichment and the consequent eutrophication of coastal waters (Nixon, 1995; Paerl, 1997; Anderson et al., 2002; Zhou et al., 2003; Heisler et al., 2008; Howarth, 2011). Although widespread eutrophication is increasing in global coastal waters, *Phaeocystis* blooms only occur in some euthrophic areas (Garrison et al., 1987; Lancelot et al., 1987; Al-Hasan et al., 1990; Chen et al., 1999), which also have large differences in other environmental factors. It is therefore necessary to study the ecological characteristics of this species, isolated from different geographic areas of the world, to explore their occurrence mechanisms and the potential developing trend of the widespread blooms. We hypothesize that *P. globosa* populations occurring in Chinese coastal waters may belong to a new ecotype, and possess a competitive advantage in their environmental adaptation over other phytoplankton species, and thus can dominate and form blooms. In this study, we examined the effects of temperature, salinity, irradiance and nutrients on the growth of a strain of *P. globosa*, isolated from the South China Sea, under nutrient-replete laboratory conditions.

2 MATERIAL AND METHOD

2.1 Culture and culturing conditions

The strain of *P. globosa* used in the study was isolated from Junk Bay, Hong Kong in 1999. Phylogenetic analysis of its partial sequence of 18S rDNA indicated that this strain is *P. globosa* (Chen et al., 2003).

Artificial seawater (salinity 30.5; Harrison et al., 1980) enriched with silicate-free f/2 culture medium (Guillard, 1975) was used as the culture medium. Before the experiments, the culture was maintained at 24°C and an irradiance of 120 μmol/(m^2·s) in a 12-h:12-h (light:dark) photoperiod cycle.

2.2 Experiments for the effect of irradiance

The irradiance experiments were conducted in an artificial climate incubator (CC275TL2H, Hangzhou, China) following the method of Kim et al. (2004). The pre-culture lasted for 1 week at 24°C, and 120 μmol/(m^2·s) from cool-white fluorescent bulbs with a 12-h light: 12-h dark cycle. The culture was inoculated with ~5 000 cells/mL in triplicate, in 50-mL capped test tubes (ø25 mm×150 mm) containing 35 mL culture suspended in f/2 modified medium, and they were gently shaken twice daily. Eleven different irradiance levels (2, 4, 7, 15, 30, 60, 90, 110, 140, 170 and 230 μmol/(m^2·s)) were obtained by wrapping the tubes with UV-absorbing vinyl screens.

A quantum light meter (Model LI-190SA; LI-COR Biosciences, Lincoln, NE, USA) was used to confirm the irradiance levels, by wrapping the receiver sensor with UV-absorbing vinyl screens. The experiment continued for one to two weeks. Every 1–2 days, an aliquot of 100 μL of culture was sampled from each test tube and cell numbers were determined using a Sedgwick-Rafter counting chamber under a compound light microscope. A fluorometer (Model TD-700; Turner Designs Co., CA, USA) was used to determine in vivo chlorophyll *a*. The specific growth rates (μ, /d) of cultures in the exponential growth phase were calculated according to the method of Guillard (Guillard, 1973), by the least-squares fit of a straight line to the growth data logarithmically transformed.

Equation 1, modified from Lederman and Tett (1981), was used to describe the relationship between growth rate and irradiance:

$$\mu=\mu_m(I-I_0)/(I+K_s-2I_0), \quad (1)$$

where μ is the specific growth rate (/day), μ_m the maximum specific growth rate (/day), I the irradiance (μmol/(m^2·s)), I_0 the compensation irradiance (μmol/(m^2·s)), and K_s the irradiance at $\mu_m/2$ (half-saturation light intensity).

2.3 Experiments for the effects of temperature and salinity

The growth experiments were conducted using a crossed factorial design with 42 different combinations of six temperatures (10, 15, 20, 24, 27, 31°C) and seven salinities (10, 15, 20, 25, 30, 35, 40) under a constant light intensity (about 120 μmol/(m^2·s)). The f/2 media with salinities < 30.5 were prepared by diluting the initial artificial seawater (salinity 30.5) with de-ionized water, and the f/2 media with salinities >30.5 were prepared by evaporating the initial artificial seawater to the desired salinities. To avoid salinity and temperature shocks, cultures were acclimated to the desired experimental conditions by stepwise transferring over 1–4 weeks according to the method outlined by Yamaguchi and Honjo (1989) and Kim et al. (2004). If the cells could not grow under an experimental regime, no further growth experiments were carried out at that particular combination of temperature and salinity, and the growth rate was regarded as zero.

For each experimental regime, acclimated stock cultures were inoculated into triplicate capped test tubes (ø25 mm×150 mm) containing 35 mL of modified f/2 medium (Guillard, 1975) without silicate. Growth rates during the exponential growth phase were calculated as described above.

2.4 Experiments for the combined effects of nutrient composition and irradiance

To clarify the combined effects of irradiance and nutrient composition on the growth of *P. globosa*, exponential-phase algal cells were inoculated into 50 mL capped test tubes (ø25 mm×150 mm) containing 35 mL culture suspended in modified f/2 medium with different DIN/DON ratios (1:4, 2:3, 3:2, 4:1). NO_3^- and urea were used as inorganic or organic nitrogen sources, respectively, and whole NO_3^- and whole urea treatments were used as controls. The total nitrogen was 300 μg/L. The experiment was conducted in an artificial climate incubator at 24°C under both high (100 μmol/(m^2·s)) and low irradiance levels (5 μmol/(m^2·s)) with a 12-h light:12-h dark cycle, using the same method as outlined in Section 2.2. All treatments were conducted in triplicate and lasted 13 d. Prior to the experiment, *P. globosa* cultures were acclimated to the corresponding nitrogen composition and irradiance by at least three transfers. A fluorometer (Model TD-700; Turner Designs Co., CA, USA) was used to determine in vivo chlorophyll *a* everyday. The specific growth rates (μ, /d) of cultures was calculated according to the Eq.2.

$$\mu=\ln(N_t/N_0)/T, \quad (2)$$

where μ is the specific growth rate (/day), N_t the fluorescence value at time t, N_0 the initial fluorescence value, and T the incubation time.

2.5 Statistical analysis

The software SPSS 11.5 for windows (SPSS, Chicago, IL, USA) was used for the analysis of variance (ANOVA), to determine the effects of temperature and salinity on growth rate. Cubic polynomial equations were determined based on the ANOVA results (Yamaguchi and Honjo, 1989; Yamaguchi et al., 1991, 1997; Ellegaard et al., 1993; Kim et al., 2004; Nagasoe et al., 2006; Matsubara et al., 2007; Xu et al., 2010).

3 RESULT

3.1 Effect of irradiance on the growth of *P. globosa*

During the experiment, no colonies were found and *P. globosa* cells remained in the solitary flagellate morphology. A specific *P. globosa* growth rate of

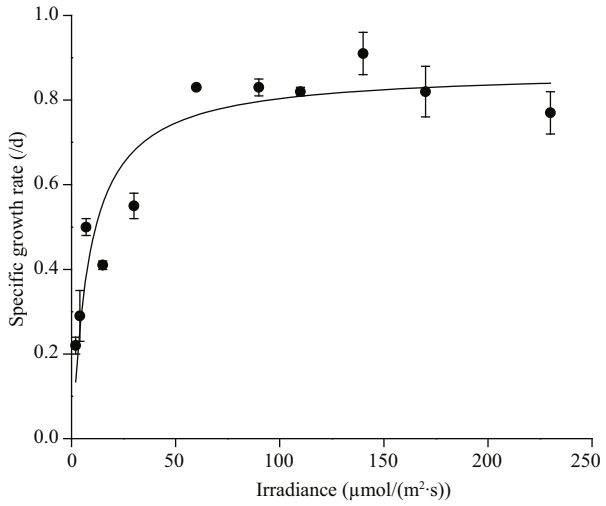


Fig.1 Specific growth rates (/d) of *P. globosa* as a function of irradiance level

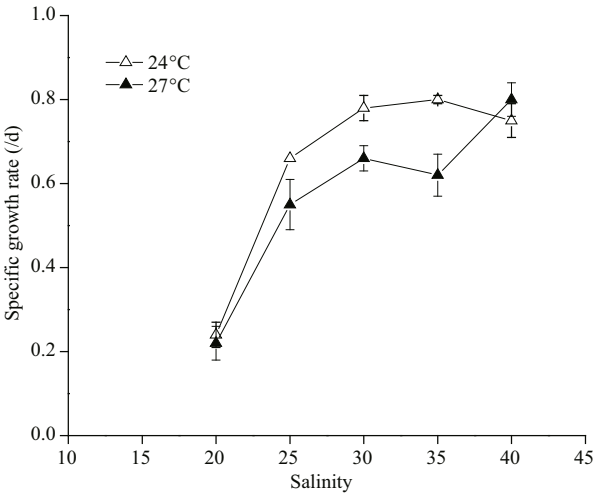


Fig.3 Specific growth rates (/d) of *P. globosa* as a function of salinity at temperatures 24°C and 27°C

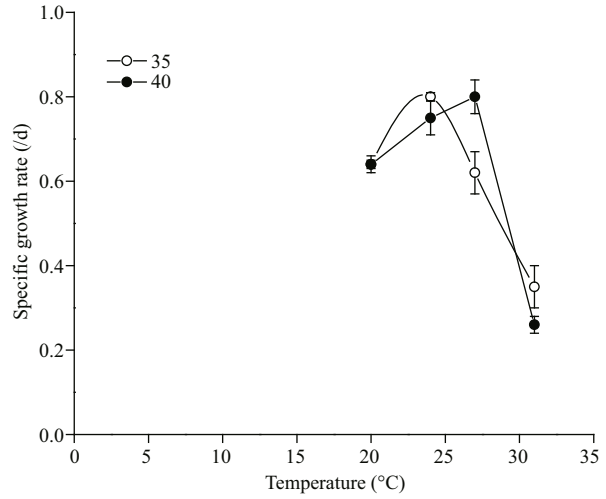


Fig.2 Specific growth rates (/d) of *P. globosa* as a function of temperature at salinities of 35 and 40

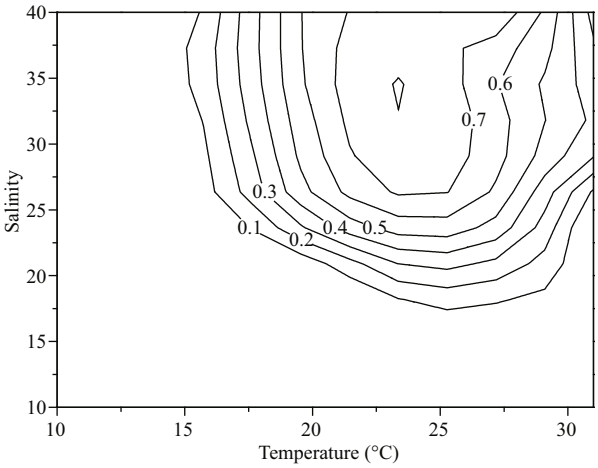


Fig.4 Contour plots for specific growth rates (/d) of *P. globosa*, as a function of temperature and salinity, from 42 combinations

~0.22/d was maintained at the lowest irradiance level of 2 μmol/(m²·s), while the growth of this organism appeared to become saturated at 60 μmol/(m²·s) with a growth rate 0.83/d (Fig.1). Photo-inhibition was not observed at an irradiance of 230 μmol/(m²·s), the maximum irradiance used in this study. Thus, the optimum irradiance for growth was ≥60 μmol/(m²·s).

The following hyperbolic equation could be used to describe the exponential growth phase, according to the data shown in Fig.1:

$$\mu=0.87(I-0.5)/(I+7.73)\ (R^2=0.919), \quad (3)$$

The compensation irradiance (I_0) was 0.5 μmol/(m²·s). The maximum growth rate (μ_m) and half-saturating irradiance (K_s) were 0.87/d and 8.73 μmol/(m²·s), respectively.

3.2 effect of temperature and salinity on the growth of *P. globosa*

Under experimental conditions, *P. globosa* existed as solitary flagellate cells. The dependence of the specific growth rate of *P. globosa* on temperature and salinity is plotted in Figs.2–4. There was no growth potential under temperatures ≤15°C at any salinity, while *P. globosa* was able to grow at temperatures between 20°C and 31°C, depending on the salinity. A maximum growth rate of 0.80/d was obtained at the following combinations: 24°C and 35, 27°C and 40. In addition, specific growth rates >0.60/d occurred when the combinations of temperature and salinity were 20°C to 27°C and 25 to 40, respectively. The range of salinities conducive to growth tended to be

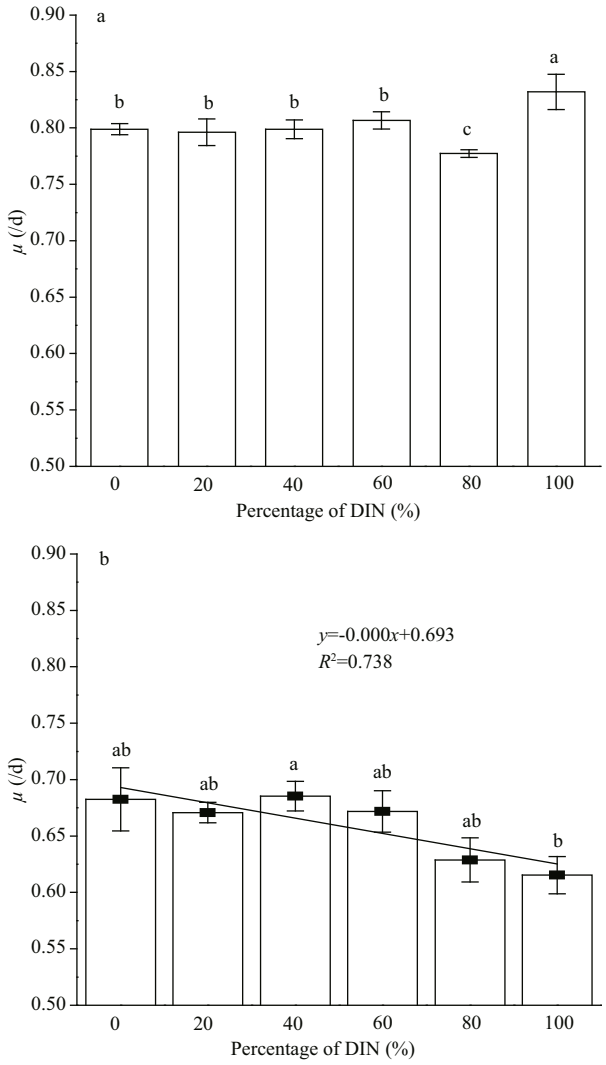


Fig.5 Specific growth rates (/d) of *P. globosa* as a function of the percentage of DIN

a. high light intensity; b. low light intensity.

narrower when temperatures were lower than 24°C or higher than 27°C (Fig.4).

Results from two-way ANOVA indicated significant effects of temperature, salinity and the interaction of temperature and salinity on the growth rates of *P. globosa* (P<0.001; Table 1). Of the total sum of squares, 38%, 37% and 24% were accounted for by the sum of squares for temperature, salinity and the temperature-salinity interaction, respectively. This indicated that, within the ranges of temperature and salinity applied in this study, the contributions of temperature and salinity to growth were approximately equal and there was a significant interaction between these two factors.

Based on the ANOVA results, a cubic equation could be fitted in a form as shown in Eq.4:

Table 1 Summary of two-way ANOVA of growth rates of *P. globosa* as a function of temperature, salinity and their interaction

Source of variation	d.f.	Sum of squares	Mean square	F
Temperature	5	4.199	0.840	1 567.174*
Salinity	6	4.100	0.683	1 275.160*
Temperature×salinity	30	2.615	0.087	162.683*
Error	84	0.045	0.001	
Total	125	10.958		

* P<0.001.

$$\mu=b_{00}+b_{10}T+b_{01}S+b_{11}TS+\cdots+b_{30}T^3+b_{03}S^3, \quad (4)$$

where μ is the specific growth rate, T the temperature, S the salinity, and b_{nn} the regression coefficients, fitted by means of the stepwise forward regression method (SPSS11.5). The multiple regression of the specific growth rate of *P. globosa* on temperature and salinity is shown as follows:

$$\mu=-3.26927+0.133831T+0.006572S+0.00608TS-0.00013T^3-0.000093TS^2+0.00000137S^3 \ (R^2=0.949). \quad (5)$$

Indeed, in situ growth rates of alga were influenced by complicated components, including irradiance, nutrients, and grazing pressure. Given suitable light, nutrients and other conditions suitable for the growth of *P. globosa*, this regression equation provides a useful reference to estimate in situ growth rates of *P. globosa* based on available temperature and salinity data.

3.3 Combined effects of nutrient composition and irradiance on the growth of *P. globosa*

Our results showed that the growth rates of *P. globosa* were significantly higher at high light intensity than at low light intensity, suggesting that irradiance was one of the key factors influencing nitrogen utilization. More specifically, the specific growth rates (/d) of *P. globosa* increased with the increase in DIN percentage composition at high light intensity (Fig.5a), and the maximum growth rate of 0.83/d was obtained in the 100% DIN treatment. At low light intensity (5 μmol/(m^2·s)), a maximum growth rate (~0.70/d) of *P. globosa* was observed in the whole-urea treatment. Statistical analysis showed that growth rate had a significant inverse relationship with increasing DIN percentage composition (Fig.5b). Our results suggested that *P. globosa* preferred organic nitrogen when ambient light intensity was low.

Table 2 Summary of irradiance (μmol/(m²·s)), temperature (°C) and salinity characteristics reported for HAB species

HAB species	I_0	I_s	Optimum temperature	Optimum salinity	References
Phaeocystis globosa	0.5	60	24–27	35–40	This study
Prorocentrum donghaiense	0.1	30	27	30	Xu et al., 2010
Pseudo-nitzschia pungens	0.1	90	27	20	Authors' unpublished data
Karenia mikimotoi	0.7	110	25	25	Yamaguchi and Honjo, 1989
Chattonella antiqua	10.3	110	25	25	Yamaguchi et al., 1991
Chattonella marina	10.5	110	25	20	Yamaguchi et al., 1991
Alexandrium tamarence	76	90	15	30	Yamamoto and Tarutani, 1997
Cochlodinium polykrikoides	10.38	90	25	34	Kim et al., 2004
Gyrodinium instriatum	10.61	70	25	30	Nagasoe et al., 2006
Akashiwo sanguinea	14.4	114	25	20	Matsubara et al., 2007

4 DISCUSSION

Eutrophication and other anthropogenic alterations have been linked to global increases in HAB frequency and intensity in the past few decades (Paerl, 1997; Anderson et al., 2002; Zhou et al., 2003; Heisler et al., 2008; Fu et al., 2012). *Phaeocystis globosa* has been one of the most important HAB causative species, causing severe damage to fisheries, tourism and aquatic ecosystems in Chinese coastal waters, and arousing great public attention (Chen et al., 1999; Wang et al., 2007; Liu et al., 2010). Our laboratory experiments also revealed that *P. globosa* required mid-nitrogen levels (half saturation constant of NO_3^-, 1.71 μmol N/L) and high phosphorus levels (half saturation constant of PO_4^{3-}, 1.08 μmol P/L), and was adapted to eutrophic waters (unpublished data, authors). *Phaeocystis globosa* blooms usually occurs at low to medium temperatures in coastal waters (Riegman and van Boekel, 1996; Schoemann et al., 2005). However, in China, a bloom spread from the Bohai Sea to the South China Sea, bloomed in different seasons and lasted a long period, particularly in the South China Sea (Wang et al., 2007). The population in Chinese coastal waters must therefore have unique ecological characteristics that allows it to adapt to variable environments. From our laboratory experiments, we confirmed that the *P. globosa* strain from the South China Sea not only prefers higher temperature and higher salinity, but is also adapted to lower irradiance levels.

Irradiance has been suggested to influence cell morphology of *P. globosa*; flagellate cells are better competitors for light than colonial cells, owing to their superior uptake characteristics (Riegman and Boekel, 1996). During the irradiance experiments, *P. globosa* was present as solitary cells. Our study showed the compensation irradiance (I_0) of *P. globosa* was 0.5 μmol/(m²·s), which is similar to *Karenia mikimotoi* (Yamaguchi and Honjo, 1989), *Prorocentrum donghaiense* (Xu et al., 2010), and *Pseudo-nitzschia pungens* (author, unpublished) (Table 2), which are representative HAB causative species in the coastal waters of China in the spring. The saturating irradiance (I_s) of *P. globosa* was 60 μmol/(m²·s), while the saturating irradiances for most HAB species were found to be higher than 90 μmol/(m²·s). This relatively lower I_s suggested that *P. globosa* may become dominant under turbid environments, where solar irradiance is attenuated rapidly (Table 2). However, photo-inhibition was not observed at the highest irradiance used in this study (230 μmol/(m²·s)), suggesting a wide tolerance range of irradiance for *P. globosa*.

Field investigations indicated that *Phaeocystis* species could survive from the sea surface to 150-m-depth water layer, exhibiting its extraordinary flexibility in response to light environments (El-Sayed et al., 1983). Further studies have indicated that it could adapt to a wide irradiance range of 16–1 600 μmol/(m²·s) (Palmisano and Sullivan, 1985). It has been reported that *Phaeocystis* can absorb blue-green light to adapt to low irradiance, and form amylase-containing colonies to protect the cells from the damage of UV-B under high light intensity (SooHoo et al., 1987). Therefore, the ability to transform between flagellate cells and colonies is a superior competing strategy for irradiance. The unique adaptation to extremely low- or high-light environments enables *Phaeocystis* to develop blooms under turbid environments in the spring, and strong irradiance in the summer.

Light is thought to be one of the most important factors influencing the photosynthesis of

phytoplankton. Further, as the main driver of phytoplankton growth in turbid estuaries, its availability would also affect nutrient utilization for phytoplankton (Harris and Lott, 1973; Chang et al., 1992; Domingues et al., 2011). An in situ incubation experiment on three size components, micro- (20–200 μm), nano- (2–20 μm) and pico-plankton (<2 μm), showed that nitrate (NO_3^-) uptake was most light-sensitive, followed by ammonium (NH_4^+) and urea (Chang et al., 1992). *Phaeocystis globosa* also had higher affinity for urea than *Scrippsiella trochoidea* or *Skeletonema costatum* at 120 μmol/(m^2·s) (Hu et al., 2010). In our culture experiments, when different DON (as urea)/DIN (as NO_3^-) ratios were provided, the corresponding specific growth rates was always higher at high light intensity (100 μmol/(m^2·s)) than at low light intensity (5 μmol/(m^2·s)). Meanwhile, at low light intensity, the specific growth rates were significantly higher with higher DON ratios than lower DON ratios (Fig.5). Thus, it can be suggested that irradiance is one of the key factors influencing nitrogen utilization, and *P. globosa* possess a flexible nutrient strategy adapting to variable light conditions.

Phaeocystis globosa was able to grow within the ranges of 20–31°C and salinities of 20–40, and the maximum growth rate (0.80/d) was obtained at two combinations, 24°C, 35 and 27°C, 40. Specific growth rates >0.60/d (75% of the maximum growth rate) were observed at 20–27°C and salinities of 25–40 (Fig.4). It appears that this strain of *P. globosa* prefers higher water temperature and salinity, whereas lower temperatures (≤15°C) and salinities (≤15) are unfavorable. Field evidence revealed that in situ temperature and salinity during *P. globosa* blooms in the South China Sea were both significantly higher than usual (Chen et al., 1999; Xu et al., 2003), which was consistent with our culture experiment. The first *P. globosa* bloom recorded in Zhelin Bay, Guangdong Province in October 1997, was associated with abnormally elevating water temperature (18–21°C) and salinity (30–33), while the average water temperature during the bloom in the summer of 1999 in the same area was 25–26°C (Xu et al., 2003). Therefore, the ranges of temperature and salinity observed during the *P. globosa* blooms fall into the ranges for optimal growth observed in our laboratory experiments (Fig.4). *Phaeocystis globosa* blooms have been observed from temperate to tropical regions and could grow at temperatures of 4–22°C, with an optimum range of 4–15°C (Grimm & Weisse, 1985; Jahnke, 1989; Jahnke & Baumann, 1987; Lancelot et al., 1991; Medlin et al., 1994; Riegman and Van Boekel, 1996; Schoemann et al., 2005). It seems that the population in the South China Sea exhibits different temperature requirements. The consistency between our experimental results and the field observations in which *P. globosa* favors higher temperature and higher salinity, suggests that the *P. globosa* population from the South China Sea is a separate ecotype.

The unique characteristics of *P. globosa*, adapting to high temperature and high salinity, implies a higher probability in developing blooms under high-temperature and low-precipitation conditions. As for the coastal waters of the Guangdong Province, the annual average temperature is around 21°C, with monthly average temperatures of above 20°C from May to November, during which the temperature is suitable for the growth of *P. globosa*. Therefore, it was not surprising that *P. globosa* blooms in the South China Sea were frequent and of a long duration (Bulletin of Marine Environmental Quality of China, 2000–2008, State Oceanic Administration, China). Furthermore, the growth of this organism requires high levels of nutrients, especially phosphorus, and its maximum growth rate is much higher than those of most other HAB species. Thus, along with eutrophication and global warming, the potential risk of *P. globosa* bloom in coastal waters of China will persist for a long period of time.

Some models have been used to identify blooms and investigate the dominant factors controlling phytoplankton production (Carstense et al., 2007; Camacho et al., 2015). Owing to differences in geographic environments and algal bloom causative species, these models should be adjusted to the specific situations. In this study, we provide a useful regression equation (Eq.5) to estimate in situ growth rates of *P. globosa*, based on temperature and salinity data available. This equation can also be used in the prevention and control of *P. globosa* blooms. In addition, nutrients are also important factors affecting the formation of blooms. In further studies, a more comprehensive model, including nutrients, should enable more accurate predictions and control of *P. globosa* blooms.

5 CONCLUSION

Our study demonstrates the ability of *P. globosa* isolated from the South China Sea to acclimate at higher temperatures, higher salinities and to lower light environments. Therefore, the Chinese population

should belong to a separate ecotype, and there is a high risk of *P. globosa* blooms developing in the South China Sea. It is important to quantify the relationship between the growth rate of HAB species and factors such as temperature and salinity, to enable understanding of the outbreak timing of harmful algal blooms. The regression equation (Eq.5) obtained in the study can be used to estimate growth rates and predict the occurrence of blooms, based on in situ water temperature and salinity data. The present study provides important information for understanding the formation mechanisms of *P. globosa* blooms in the coastal waters of China, and for developing techniques to predict the occurrence of blooms in the field.

References

Al-Hasan R H, Ali A M, Radwan S S. 1990. Lipids, and their constituent fatty acids, of *Phaeocystis* sp. from the Arabian Gulf. *Mar. Biol*., **105**(1): 9-14.

Anderson D M, Glibert P M, Burkholder J M. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*, **25**(4): 704-726.

Camacho R A, Martin J L, Watson B, Paul M, Zheng L, Stribling J B. 2015. Modeling the factors controlling phytoplankton in the St. Louis Bay estuary, Mississippi and evaluating estuarine responses to nutrient load modifications. *Journal of Environmental Engineering*, **141**(3), http://dx.doi.org/10.1061/(ASCE)EE.1943-7870.0000892.

Carstensen J, Henriksen P, Heiskanen A S. 2007. Summer algal blooms in shallow estuaries: definition, mechanisms, and link to eutrophication. *Limnology and Oceanography*, **52**(1): 370-384.

Chang F H, Vincent W F, Woods P H. 1992. Nitrogen utilization by size-fractionated phytoplankton assemblages associated with an upwelling event off Westland, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, **26**(3-4): 287-301.

Chen J F, Xu N, Jiang T J, Wang Y, Wang Z H, Qi Y Z. 1999. A report of *Phaeocystis globosa* bloom in coastal water of Southeast China. *Journal of Ji'nan University* (*Natural Science & Medicine Edition*), **20**(3): 124-129. (in Chinese with English abstract)

Chen L F, Zhang Q, Luo Y M, Han B P. 2003. Molecular identification of the Hong Kong strain P_2 of *Phoeocystis* as *Phaeocystis globosa*. *Ecologic Science*, **22**(4): 349-350. (in Chinese with English abstract)

Chen Y Q, Wang N, Zhang P, Zhou H, Qu L H. 2002. Molecular evidence identifies bloom-forming *Phaeocystis* (Prymnesiophyta) from coastal waters of southeast China as *Phaeocystis globosa*. *Biochemical Systematics and Ecology*, **30**(1): 15-22.

Domingues R B, Anselmo T P, Barbosa A B, Sommer U, Galvão H M. 2011. Light as a driver of phytoplankton growth and production in the freshwater tidal zone of a turbid estuary. *Estuarine, Coastal and Shelf Science*, **91**(4): 526-535.

Ellegaard M, Christensen N F, Meostrup ∅. 1993. Temperature and salinity effects on growth of a non-chain-forming strain of *Fymnodinium catenatum* (Dinoohyceae) established from a cyst from recent sediments in the Sound (∅resund), Denmark. *J. Phycol*., **29**(4): 418-426.

El-Sayed S Z, Biggs D C, Holm-Hansen O. 1983. Phytoplankton standing crop, primary productivity, and near-surface nitrogenous nutrient fields in the Ross Sea, Antarctica. *Deep Sea Research Part A. Oceanographic Research Papers*, **30**(8): 871-886.

Fu F X, Tatters A O, Hutchins D A. 2012. Global change and the future of harmful algal blooms in the ocean. *Marine Ecology Progress Series*, **470**: 207-233.

Garrison D L, Buck K R, Fryxell G A. 1987. Algal assemblages in Antarctic pack ice and in ice-edge plankton. *J. Phycol*., **23**(4): 564-572.

Grimm N, Weisse T. 1985. Die Temperaturabhängigkeit des Wachstums von *Phaeocystis pouchetii* (Haptophyceae) in Batchkulturen. *Helgoländer Meeresuntersuchungen*, **39**(2): 201-211.

Guillard R R L. 1973. Division rates. *In*: Stein J R ed. Handbook of Phycological Methods. Culture Methods and Growth Measurements. Cambridge University Press, Cambridge. p.289-311.

Guillard R R L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In*: Smith W L, Chanley M H eds. Cultures of Marine Invertebrate Animals. Plenum Press, New York. p.29-60.

Harris G P, Lott J N A. 1973. Light intensity and photosynthetic rates in phytoplankton. *Journal of the Fisheries Research Board of Canada*, **30**(12): 1 771-1 778.

Harrison P J, Waters R E, Taylor F J R. 1980. A broad spectrum artificial sea water medium for coastal and open ocean phytoplankton 1. *Journal of Phycology*, **16**(1): 28-35.

He J W, Shi Z X, Zhang Y H, Liu Y D, Jiang T J, Yin Y W, Qi Y Z. 1999. Morphological characteristics and toxins of *Phaeocystis* cf. *pouchetii* (prymnesiophyceae). *Oceanologia et Limnologia Sinica*, **30**(2): 172-179. (in Chinese with English abstract)

Heisler J, Glibert P M, Burkholder J M, Anderson D M, Cochlan W, Dennison W C, Dortch Q, Gobler C J, Heil C A, Humphries E, Lewitus A, Magnien R, Marshallm H G, Sellner K, Stockwell D A, Stoecker D K, Suddleson M. 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, **8**(1): 3-13.

Howarth R, Chan F, Conley D J, Garnier J, Doney S C, Marino R, Billen G. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. *Frontiers in Ecology and the Environment*, **9**(1): 18-26.

Hu Z X, Xu N, Duan S S, Li A, Zhang C W. 2010. Effects of urea on the growth of *Phaeocystis globosa*, *Scrippsiella trochoidea*, *Skeletonema costatum*. *Acta Scientiae Circumstantiae*, **30**(6): 1 265-1 270. (in Chinese with English abstract)

Jahnke J, Baumann M E M. 1987. Differentiation between

Phaeocystis pouchetii (Har.) Lagerheim and *Phaeocystis globosa* Scherffel I. Colony shapes and temperature tolerance. *Hydrobiol*. *Bull*., **21**(2): 141-147.

Jahnke J. 1989. The light and temperature dependence of growth rate and elemental composition of *Phaeocystis globosa* scherffel and *P*. *pouchetii* (Har.) Lagerh. in batch cultures. *Neth*. *J*. *Sea Res*., **23**(1): 15-21.

Kim D I, Matsuyama Y, Nagasoe S, Yamaguchi M, Yoon Y H, Oshima Y, Imada N, Honjo T. 2004. Effects of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate *Cochlodinium polykrikoides* Margalef (Dinophyceae). *J*. *Plankton*. *Res*., **26**(1): 61-66.

Lancelot C, Billen G, Sournia A, Weisse T, Colijn F, Veldhuis M J W, Davies A, Wassmann P. 1987. *Phaeocystis* blooms and nutrient enrichment in the continental coastal zones of the North Sea. *Ambio*, **16**(1): 38-46.

Lancelot C, Veth C, Mthot S. 1991. Modelling ice-edge phytoplankton bloom in the Scotia-Weddell sea sector of the Southern Ocean during spring 1988. *Journal of Marine Systems*, **2**(3-4): 333-346.

Lederman T C, Tett P. 1981. Problems in modelling the photosynthesis-light relationship for phytoplankton. *Bot*. *Mar*., **24**(3): 125-134.

Liu J S, van Rijssel M, Yang W D, Peng X C, Lu S H, Wang Y, Chen J F, Wang Z H, Qi Y Z. 2010. Negative effects of *Phaeocystis globosa* on microalgae. *Chinese Journal of Oceanology and Limnology*, **28**(4): 911-916.

Matsubara T, Nagasoe S, Yamasaki Y, Shikata T, Shimasaki Y, Oshima Y, Honjo T. 2007. Effects of temperature, salinity, and irradiance on the growth of the dinoflagellate *Akashiwo sanguinea*. *Journal of Experimental Marine Biology and Ecology*, **342**(2): 226-230.

Medlin L K, Lange M, Baumann M E M. 1994. Genetic differentiation among three colony-forming species of *Phaeocystis*: further evidence for the phylogeny of the Prymnesiophyta. *Phycologia*, **33**(3): 199-212.

Nagasoe S, Kim D I, Shimasaki Y, Oshima Y, Yamaguchi M, Honjo T. 2006. Effects of temperature, salinity and irradiance on the growth of the red tide dinoflagellate *Gyrodinium instriatum* Freudenthal et Lee. *Harmful Algae*, **5**(1): 20-25.

Nixon S W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, **41**(1): 199-219.

Paerl H W. 1997. Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as 'new' nitrogen and other nutrient sources. *Limnology and Oceanography*, **42**(5 Part 2): 1 154-1 165.

Palmisano A C, Sullivan C W. 1985. Pathways of photosynthetic carbon assimilation in sea-ice microalgae from McMurdo Sound, Antarctica. *Limnology and Oceanography*, **30**(3): 674-678.

Qi Y Z, Chen J F, Wang Z H, Xu N, Wang Y, Shen P P, Lu S H, Hodgkiss I J. 2004. Some observations on harmful algal bloom (HAB) events along the coast of Guangdong, southern China in 1998. *Hydrobiologia*, **512**(1-3): 209-214.

Riegman R, Van Boekel W. 1996. The ecophysiology of *Phaeocystis globosa*: a review. *Journal of Sea Research*, **35**(4): 235-242.

Schoemann V, Becquevort S, Stefels J, Rousseau V, Lancelot C. 2005. *Phaeocystis* blooms in the global ocean and their controlling mechanisms: a review. *J*. *Sea Res*., **53**(1): 43-66.

Shen P P, Wang Y, Qi Y Z, Xie L C, Lü S H, Hodgkiss I J. 2000. Growth characteristics and life cycle of *Phaeocystis globosa* Scherffel. *Acta Hydrobiologica Sinica*, **24**(6): 635-643. (in Chinese with English abstract)

SooHoo J B, Palmisano A C, Kottmeier S T, Lizotte M P, SooHoo S L, Sullivan C W. 1987. Spectral light absorption and quantum yield of photosynthesis in sea ice microalgae and a bloom of *Phaeocystis pouchetii* from McMurdo Sound, Antarctica. *Mar*. *Ecol*. *Prog*. *Ser*., **39**: 175-189.

Sunda W G, Graneli E, Gobler C J. 2006. Positive feedback and the development and persistence of ecosystem disruptive algal blooms. *Journal of Phycology*, **42**(5): 963-974.

Wang N, Chen Y Q, Qu L H, Li T H, Qi Y Z. 2000. Analysis of 18S rDNA gene from a "red tide" related *Phaeocystis* species in South China Sea. *Acta Scientiarum Naturalium Universitatis Sunyatseni*, **39**(1): 127-128. (in Chinese with English abstract)

Wang Y, Qi Y Z, Li S S. 2007. Nutritional requirements for the growth of *Phaeocystis globosa* Scherffel. *Acta Hydrobiologica Sinica*, **31**(1): 24-29. (in Chinese with English abstract)

Xu N, Duan S S, Li A F, Zhang C W, Cai Z P, Hu Z X. 2010. Effects of temperature, salinity and irradiance on the growth of the harmful dinoflagellate *Prorocentrum donghaiense* Lu. *Harmful Algae*, **9**(1): 13-17.

Xu N, Qi Y Z, Chen J F, Huang W J, Lu S H, Wang Y. 2003. Analysis on the cause of *Phaeocystis globosa* Scherffel red tide. *Acta Scientiae Circumstantiae*, **23**(1): 113-118. (in Chinese with English abstract)

Yamaguchi M, Honjo T. 1989. Effects of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). *Nippon Suisan Gakkaishi*, **55**(11): 2 029-2 036. (in Japanese with English abstract)

Yamaguchi M, Imai I, Honjo T. 1991. Effects of temperature, salinity and irradiance on the growth rates of the noxious red tide flagellates *Chattonella antiqua* and *C*. *marina* (Rhaphidophyceae). *Nippon Suisan Gakkaishi*, **57**(7): 1 277-1 284. (in Japanese with English abstract)

Yamaguchi M, Shigeru I, Nagasaki K, Matsuyama Y, Uchida T, Imai I. 1997. Effects of temperature and salinity on the growth of the red tide flagellates *Heterocapsa circularisquama* (Dinophyceae) and *Chattonella verruculosa* (Raphidophyceae). *J*. *Plankton Res*., **19**(8): 1 167-1 174.

Yamamoto T, Tarutani K. 1997. Effect of temperature, salinity and irradiance on the growth of toxic dinoflagellate *Alexandrium tamarense* isolated from Hiroshima Bay, Japan. *Jpn*. *J*. *Phycol*. (*Sorui*), **45**: 95-101. (in Japanese with English abstract)

Zhou M J, Yan T, Zou J Z. 2003. Preliminary analysis of the characteristics of red tide areas in Changjiang River estuary and its adjacent sea. *Chinese Journal of Applied Ecology*, **14**(7): 1 031-1 038. (in Chinese with English abstract)