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Population dynamics of *Pseudo-nitzschia pungens* in Zhelin Bay, China

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Population dynamics of the planktonic diatom Pseudo-nitzschia pungens *was investigated at the Zhelin Bay, China, between May 2000 and December 2004. Monthly or seasonal plankton samples were collected from nine stations along the inner to outer bay gradient. Among the 1045 samples collected,* P. pungens *on average accounted for 2.9% of the total phytoplankton cells, with densities ranging from 0 to 50.94 × 10^4 cells l^{-1} and a grand mean of 1.43 × 10^4 cells l^{-1}. Two hundred and fourteen samples (20.5%) had densities of* P. pungens *above 10^4 cells l^{-1} and 40 samples (3.8%) had densities that were above 10^5 cells l^{-1}. Results of the grey incidence–regression analysis show that water temperature, zooplankton and salinity were the most important among the 13 environmental factors influencing population density of* P. pungens. *Water temperature has a highly significant linear relationship with the population density, with 23.8°C or higher being an essential condition for the algal bloom. Grazing by zooplankton was probably the most important factor controlling the algal bloom. With continued decreasing of richness and organism size of the zooplankton community at the Zhelin Bay,* P. pungens *blooms may become more frequent. The bay has a large-scale mariculture operation, therefore it is important to carefully examine and monitor the potential impacts of toxin-producing* P. pungens *on human health as well as ecosystem health of the bay.*

Keywords: Zhelin Bay, *Pseudo-nitzschia pungens*, population dynamics, algal blooms, diatoms, eutrophication

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INTRODUCTION

Pseudo-nitzschia pungens is a eurythermal and euryhaline planktonic diatom species distributed widely in coastal areas around the world (Hasle, 2002; Casteleyna *et al.*, 2008). Rhodes *et al.* (1996) reported that *P. pungens* isolated from certain coastal areas of New Zealand produced domoic acid, whereas the same species from other areas of New Zealand did not. Prior to this, *P. pungens* was considered non-toxic (Smith *et al.*, 1990; Bates *et al.*, 1993; Villac *et al.*, 1993; Wang *et al.*, 1993; Lundholm *et al.*, 1994; Villareal *et al.*, 1994; Vrieling *et al.*, 1996). However, Trainer *et al.* (2000) reported toxin-producing *P. pungens* in many other parts of the world. Since *P. pungens* is widely distributed along China's coast and a major component of the toxic algal blooms, e.g. in Dalian, Qingdao, Yellow Sea, Changjiang River mouth, Xiamen and South China Sea (Zou *et al.*, 1993; Qi *et al.*, 1996), it is important to study the physiology, population ecology, and toxicity of the species to understand the bloom-forming mechanism and develop bloom prevention and seafood safety monitoring programmes. In recent years, *Pseudo-nitzschia* spp. has been studied extensively under laboratory conditions, however, population ecology of the species in its natural environment has rarely been reported in China and worldwide. The need for rigorous field investigations of these diatom populations has been recognized repeatedly in recent literature (e.g. Bates & Trainer, 2006).

Zhelin Bay is located in south-eastern China, on the north shore of the South China Sea (Figure 1). The aquaculture operations in the bay have increased dramatically since the late 1980s, with approximately half of the water surface occupied by either oyster or cage-fish farms (Huang *et al.*, 2004). The rapid expansion of aquaculture, human population increase and inadequate sewage treatment have all contributed to the severe eutrophication conditions (e.g. high nutrients) and frequent occurrence of algal blooms (e.g. *Skeletonema costatum* and *Thalassosira diporocyclus*) in the bay, negatively impacting the fisheries and ecosystem (Chen *et al.*, 2004; Huang *et al.*, 2004). To quantify the degree of eutrophication and understand the mechanisms of harmful algal blooms, we have conducted a comprehensive survey and monitoring of environmental parameters, nutrients, plankton, microorganisms, heavy metal, and organic pollutants in the water and sediment since 2000. The present study reports the results of the population dynamics of *P. pungens*.

MATERIALS AND METHODS

Sampling stations

Nine stations along the inner to outer bay gradient were established (Figure 1). Station S_1 was located at the mouth of Huanggang River; S_2 at the Sanbaimen Port; S_3 and S_4 at the

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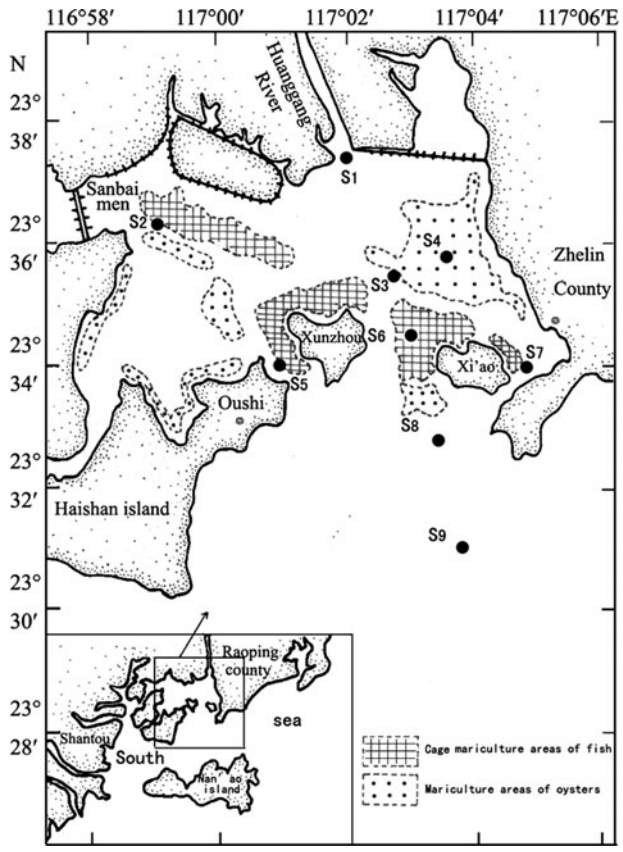

Fig. 1. Map of the sampling sites.

edge and centre of an oyster culture area (~15 km^2), respectively; S_5 and S_7 were at the edge of the fish-cage culture areas; S_6 was at the centre of a fish-cage culture area; S_8 and S_9 were on the outer bay, away from the aquaculture areas. Locating the sampling stations and calculating the culture area were accomplished with the help of a Global Positioning System (GPS12, Garmin Corporation).

Sample collection and analysis

From May 2000 to June 2001, the eight stations (except for S_3) were sampled twice per month during the winter (December to February) and 3–4 times per month during the other seasons. From July 2001 to December 2003, all the nine stations were sampled once a month. In 2004, all the nine stations were sampled once per season: spring (March to May), summer (June to August), autumn (September to November) and winter (December to February). Each sampling was accomplished around the high tide (±1.5 h). A shallow water type III plankton net (diameter of 37 cm, area of 0.1 m^2 and mesh size of 77 μm) (Administration of Technical Supervision of People's Republic of China, 1992) was pulled vertically from about 0.5 m above the bottom up to the water surface. The samples collected were fixed *in situ* with formalin to a final concentration of 4% and transported back to the laboratory for algal species identification. To quantify the algal composition and density, a Niskin bottle (HQM-1) was used to collect a 1 l water sample from the bottom (about 0.5 m above the bottom surface) and surface (about 0.5 m below the water surface). Each sample was poured into a polyethylene bottle, fixed with Lugol's solution to a final concentration of 15 and transported back to the laboratory. In the laboratory, the samples were transferred to glass beakers. Twenty-four hours later, the supernatant was repeatedly siphoned off using a meshed (77 μm) pipe until the sample volume was reduced to 30–100 ml. The sample was well mixed before a subsample (1 ml) was taken and placed into a Sedgwick–Rafter phytoplankton counter and under an inverted microscope (Zeiss, Axiovert 25). The algae were identified to the lowest taxa possible and enumerated. Zooplankton samples were collected and enumerated as described previously by Dong *et al.* (2006).

Water temperature, salinity, turbidity, dissolved oxygen (DO) and pH were measured *in situ* using a portable water quality analyser (YSI 6600-02, USA). At each station, a water sample of 250 ml was taken from surface and bottom, respectively, filtered (mesh size of 1–3 μm) and transported back to the laboratory in a cooler. A continuous flow analysis (SKALAR, Netherlands) was used to measure the total dissolved inorganic nitrogen (DIN), ammonia (NH_4-N), nitrate (NO_3-N), nitrite (NO_2-N), total dissolved inorganic phosphorous (DIP) and SiO_3-Si. The iron (Fe) and chlorophyll-*a* concentrations were measured spectrophotometrically (UV-2501PC spectrophotometer, Japan).

Data analyses

Average monthly and yearly densities of *P. pungens* at each station and average monthly density at all the stations were calculated. Because the water was relatively shallow (3–12 m) and well mixed, the surface and bottom values were averaged when calculating densities for each station. Grey incidence–regression analysis was used to evaluate the relationship between the density of *P. pungens* and the 13 environmental parameters (detailed in Lin *et al.*, 2005). The samples taken prior to September 2001 were incomplete (lacking certain environmental parameters) and therefore excluded from the analysis.

RESULTS

A total of 1045 samples were collected. Density of *P. pungens* in these samples ranged from 0 to 50.94×10^4 cells l^{-1}, with an overall average of 1.43×10^4 cells l^{-1}, accounting for 0–53.4% (average 2.9%) of the total phytoplankton density. Two hundred and fourteen samples (20.5%) had densities above 10^4 cells l^{-1} (considered harmful algal bloom for the species) and 40 samples (3.8%) had densities above 10^5 cells l^{-1}. Eighty-seven (40.7%) of the 214 samples that had densities above 10^4 cells l^{-1} and 14 (35.0%) of the 40 samples that had densities above 10^5 cells l^{-1} were found in the two outer bay stations (S_8 and S_9).

The overall average density of *P. pungens* per station during the study period ranged from 0.58×10^4 to 2.80×10^4 cells l^{-1}, with a trend of increasing from inner to outer bay, except for the low density (0.80×10^4 cells l^{-1}) at S_7 (Figure 2). There are variations among the years, with 2000 having the highest average density (5.28×10^4 cells l^{-1}) when compared to the other years and 2002 having the lowest density (0.19×10^4 cells l^{-1}). The average density of

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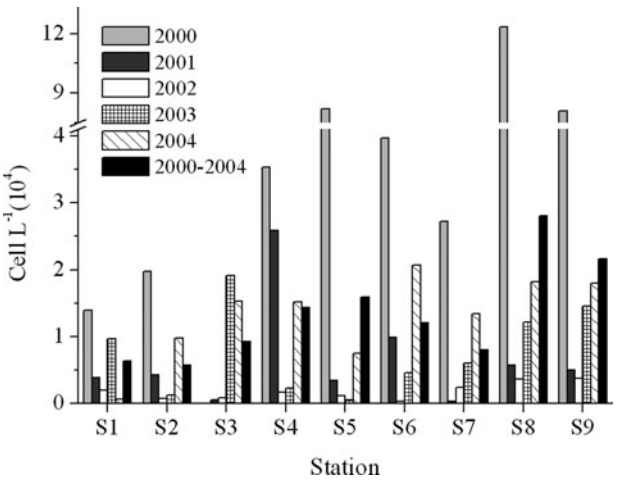

Fig. 2. The station average of *Pseudo-nitzschia pungens* during the investigation (2000–2004).

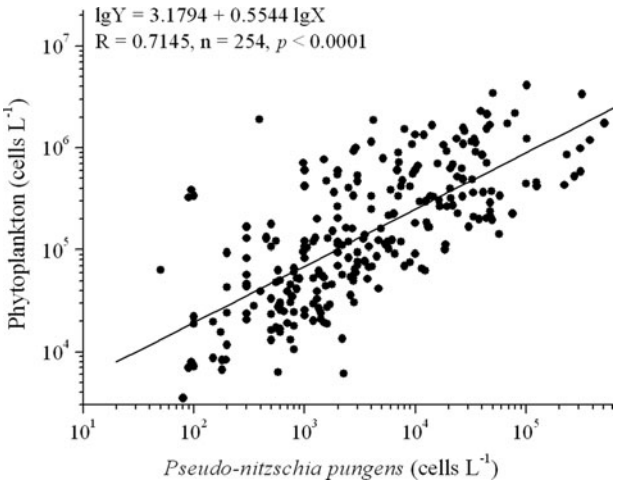

Fig. 4. Linear relationship between the densities of *Pseudo-nitzschia pungens* and total phytoplankton.

P. pungens over the whole investigation area throughout the study period was 1.43×10^4 cells l^{-1}.

Monthly average density over the study period ranged from 0 to 19.88×10^4 cells l^{-1}, with a peak during the warm (July to September) and a low during the cold months (December to April) (Figure 3). During the cold season (December to March), *P. pungens* exhibited zero population density at six sampling periods (at least once a year) (Figure 3). On the other hand, the population density was exceptionally high in January 2002 and February 2003 (Figure 3). In general, the population density of *P. pungens* exhibited a unimodal annual variation during the study period (Figure 3).

There is a highly significant linear relationship between the densities of *P. pungens* and total phytoplankton (Figure 4).

There is a significant linear relationship between the *P. pungens* density and water temperature (Figure 5). With one exception, water temperatures of the 214 samples with a density 10^4 cells l^{-1} or higher were all in the range of 23.8–30.4°C and temperatures of the 40 samples with a density of 10^5 cells l^{-1} or higher were all in a range of 29.0–30.4°C (Figure 5). The relationships between the algal density and salinity (Figure 5) and various nutrients (Figure 6), however, are not significant.

Results of the grey incidence–regression analysis show that the influence of the various environmental factors when $P = 0.1$ are: temperature > zooplankton > salinity > pH > DIN > NO_3-N > DO > DIP > NO_2-N > NH_4-N > Fe > SiO_3-Si > turbidity (Table 1).

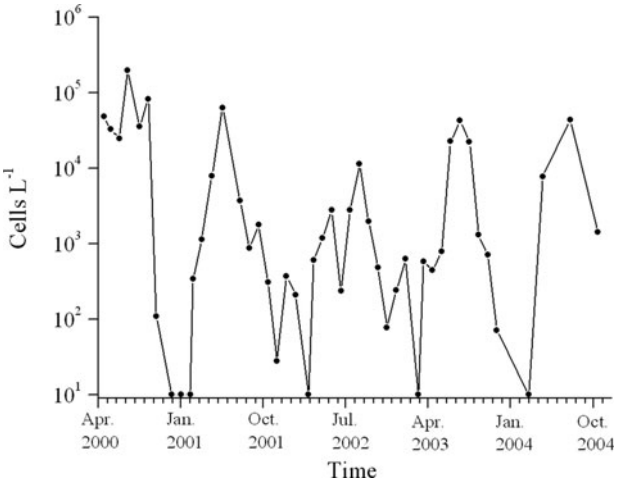

Fig. 3. The monthly average of *Pseudo-nitzschia pungens* over the investigation area.

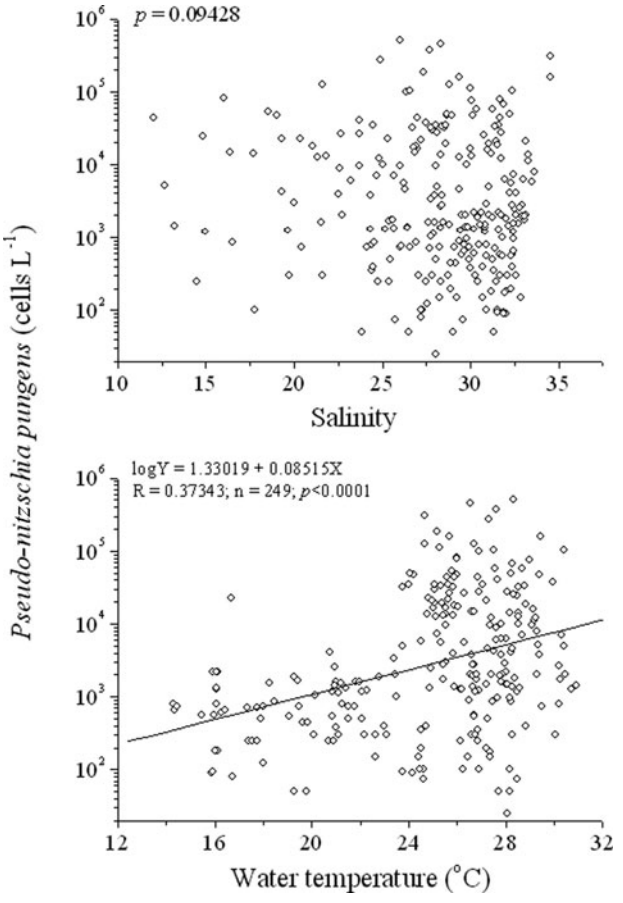

Fig. 5. The relationship between densities of *Pseudo-nitzschia pungens* and water temperature and salinity.

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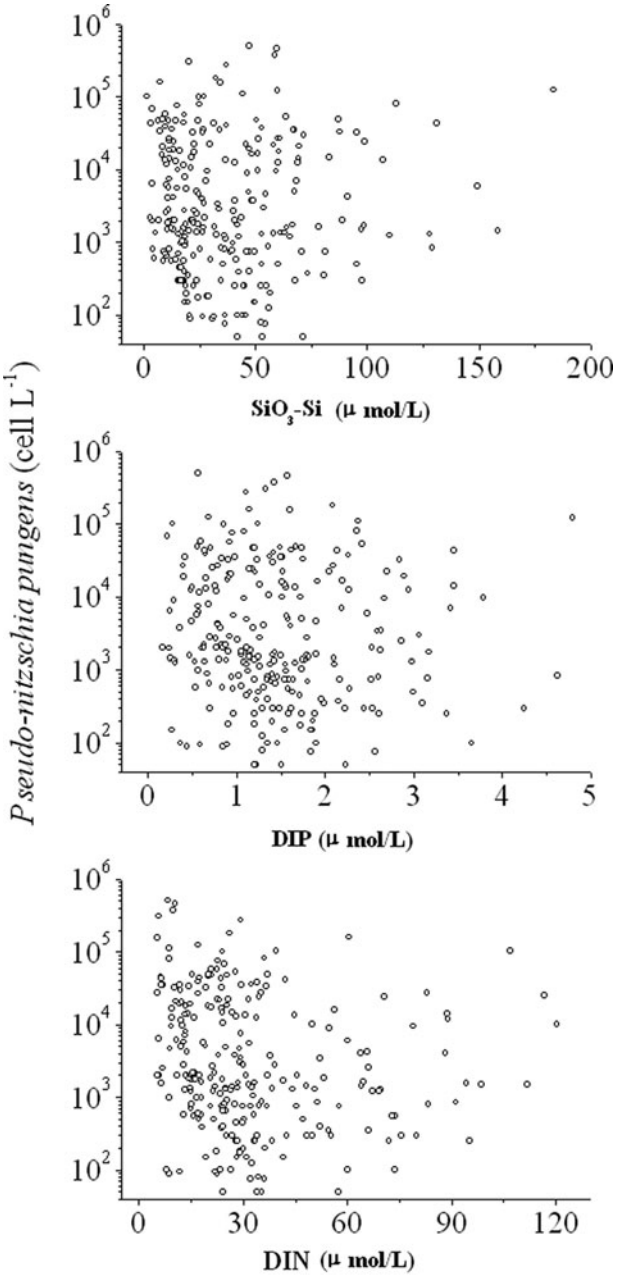

Fig. 6. The relationship between densities of *Pseudo-nitzschia pungens* and nutrients.

Table 1. Results of the grey incidence–regression analysis between *Pseudo-nitzschia pungens* and other variables.

Factor	Degree of association	Order
Temperature	0.795	1
Salinity	0.779	3
DO	0.7631	7
pH	0.773	4
Turbidity	0.744	13
Fe	0.752	11
DIP	0.758	8
SiO_3-Si	0.751	12
NO_2-N	0.757	9
NO_3-N	0.763	6
NH_4-N	0.753	10
DIN	0.767	5
Zooplankton	0.783	2

DISCUSSION

Although *P. pungens* only accounted for an average of 2.9% of the total phytoplankton cells during the study period, its grand mean density reached 1.43×10^4 cells l^{-1} and it is the second most abundant species after *Skeletonema costatum* in Zhelin Bay (Huang *et al.*, 2007). A survey at the Guanghai Bay, Nanhai, Guangdong province in July 1988 found that the population density of *P. pungens* only reached the order of 10^3 cells l^{-1} (Lu *et al.*, 1993). Another survey at the Daya Bay, also in Guangdong province, in April 1991, found that during the two *P. pungens* algal blooms, the densities were 1.82×10^4 and 3.50×10^4 cells l^{-1}, respectively (Li *et al.*, 1993). On the other hand, the total algal density reached the order of 10^6 cells l^{-1} during a harmful algal bloom of *P. pungens* and related taxa at the Amurskii Bay, Japan in June 1992 (Orlova *et al.*, 1996). Based on the cell size (length of *P. pungens* cell is about 102–134 μm) and population dynamics of the species, it was concluded that a density of 10^4–10^6 cells l^{-1} should be considered a harmful algal bloom (Smith *et al.*, 1990; Li *et al.*, 1993; Martin *et al.*, 1993). In a review of the nine harmful algal blooms of *Pseudo-nitzschia* species in North America during the 1980s and 1990s, Bates *et al.* (1998) found that seven of them had a density of 10^4–10^5 cells l^{-1} and the other two had a density of 10^6 and 10^7 cells l^{-1}. In the present study, over 20% of the samples had a *P. pungens* density of 10^4 cells l^{-1} or higher, indicating that there are possibilities for *P. pungens* blooms to occur around Zhelin Bay. Since the eutrophication is high in Zhelin Bay (Huang *et al.*, 2004) and algal blooms have occurred frequently during recent years (Huang *et al.*, 1999; Chen *et al.*, 2004), the possibility of large-scale bloom of *P. pungens* would be high if the ecosystem degenerates further or physical and chemical conditions change to favour the competitive ability of *P. pungens*.

Although *P. pungens* occurred in samples with a water temperature of 14.3–32.3°C in Zhelin Bay, it normally disappeared during the cold months each year and had a strong positive correlation with water temperature. A laboratory study also found that growth rate of *P. pungens* f. *multiseries* correlated positively and significantly with temperature in a range between 5°C and 25°C (Lewis *et al.*, 1993). Because there were excess nutrients available at Zhelin Bay, the *P. pungens* population is likely to increase exponentially if water temperature is optimal. Water temperature was unusually high during January 2002 and February 2003, and resulted in high densities of *P. pungens*, indicating that dominant phytoplankton species in eutrophic waters are mainly controlled by water temperature or non-nutrient factors (Huang *et al.*, 2007).

The present study suggests that a threshold temperature of 23.8°C was an essential condition for the bloom of *P. pungens* to occur (reached a density of 10^4 cells l^{-1} or higher). This finding agrees with many other studies conducted in North America (e.g. Taylor & Hargh, 1996; Horner *et al.*, 1997; Bates *et al.*, 1998). Water temperature at Zhelin Bay is normally higher than 23.8°C from May to October each year, when densities of other phytoplankton (e.g. *Skeletonema*) and zooplankton species are also high (Dong *et al.*, 2006). Therefore, competition and grazing might sometimes slow down the increase of *P. pungens* populations and prevent the bloom from occurring. The formation of an algal bloom is a complicated process resulting from interactions of many factors such as temperature, salinity, nutrients, competition,

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grazing and current (Keller *et al.*, 1999; Boyd *et al.*, 2000; Yang & Hodgekiss, 2003). The large-scale bloom of *Phaeocystis pouchetii* along the south-eastern coast of China (including Zhelin Bay) in November–December 1997 (Huang *et al.*, 1999) and a bloom of *Thalassiosira diporocyclus* off Zhelin Bay in December 2001 (Chen *et al.*, 2004) were probably initiated by the unusually high water temperature in the winter, as competition and grazing pressure may be low then (Yao, 2005; Dong *et al.*, 2006). The scope, season, range and duration of harmful algal blooms around the world are all showing the trend of expansion, due to global warming, eutrophication and ecological degradation (e.g. Bates & Trainer, 2006). Winter algal blooms of other species have occurred at Zhelin Bay and adjacent waters; therefore it is possible that the *P. pungens* bloom can occur there as well. A *P. pungens* bloom at the nearby Daya Bay occurred when the water temperature was only 19.7°C in April 1991 (Li *et al.*, 1993). Another *P. pungens* bloom happened at the Amurskii Bay, Japan in June 1992 when the water temperature was only 14.5°C (Orlova *et al.*, 1996).

There is a trend of increasing population density of *P. pungens* from the inner to outer bay, similar to that for the total phytoplankton density. Salinity at Zhelin Bay fluctuated between 8.96 and 35.02 and may be one of the important factors influencing the distribution and density of *P. pungens*. A survey of the *Peudo-nitzschia* spp. in the Willapa Bay, Wahsington, USA shows that the algae only reached high densities when the salinity was close to 29 (Sayce & Horner, 1996). The optimal salinity-range for growth of the *P. pungens* isolated from Nova Scottia, Canada was 15–30 (Jackson *et al.*, 1992). Therefore, although the species may live throughout Zhelin Bay, its growth may be depressed in the low salinity regions. However, since more than half of the samples with a density of 10^4 cells l^{-1} or higher were found inside of the bay and the large-scale aquaculture in the bay is mainly concentrated in the outer bay, where the salinity is 25 or higher (Dong *et al.*, 2006), a *P. pungens* bloom could occur in the large-scale mariculture areas of the bay despite the low salinity inside the bay.

Grey incidence analysis is a mathematical method that ranks the sequence of importance of various variables in a complicated system, and has been used successfully to elucidate the significant factors in ecosystems (e.g. Huang *et al.*, 2000; Lin *et al.*, 2005; Liu & Lin, 2006). The present study showed that zooplankton is the second most important factor, after temperature, in influencing the population density of *P. pungens*. This suggests that the algae are good feed for the zooplankton, a fact that has been verified in several laboratory feeding studies (Wang, 1990; Lincoln *et al.*, 2001). Because the temporal and spatial distribution of the zooplankton richness (Yao, 2005) is similar to that for the algae, the zooplankton in Zhelin Bay may be able to suppress the algal growth to a certain degree. But with the ever increasing eutrophication and pollution, the zooplankton community richness in the bay has gradually declined in recent years and its components have changed to species of smaller sizes (Dong *et al.*, 2006). The diatom *Skeletonema* accounted for an average of 67.1% of the total phytoplankton cells and may reduce the grazing pressure on *P. pungens* (Curl & McLeod, 1961; Huang *et al.*, 2007). If the trend of zooplankton community change continues, the grazing pressure may continue to decline and the probability of harmful algal blooms at Zhelin Bay may increase.

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