Temporal population dynamics of the dinoflagellate *Prorocentrum minimum* in a semi-enclosed mariculture pond and its relationship to environmental factors and protozoan grazers*

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Abstract   The ecological processes and interrelationships between protists, either autotrophic or heterotrophic, and environmental factors in mariculture ponds are largely unknown. This study investigated the temporal dynamics of the potentially harmful dinoflagellate, *Prorocentrum minimum* (Pavillard) Schiller*,* and its relationship to physico-chemical factors and protozoan grazers over a complete cycle in a semi-enclosed shrimp-farming pond near Qingdao, Northern China. *P. minimum* occurred frequently in low numbers from June to August, followed by a sharp increase from the middle of August, reaching a single maximum peak value of 2.2×10^5 cells L^{-1} in October. Temporal variation in abundance was positively correlated with dissolved nitrogen, but showed a significant inverse relationship to abundance of the dominant ciliates, *Tintinnopsis lohmanni* and *Askenasia stellaris*. The results provide statistical evidence that *P. minimum* numbers increased with increasing nitrogen, and suppression or shortening of algal bloom may be associated with protozoan grazers, such as *Tintinnopsis lohmanni,* in mariculture ponds.

Keyword: dinoflagellate; harmful algae; *Prorocentrum minimum*; shrimp-farming

1 INTRODUCTION

Prorocentrum minimum (Pavillard) Schiller is a common bloom-forming, autotrophic dinoflagellate of temperate and subtropical waters. Its ecology and bloom dynamics have been well documented from areas such as Chesapeake Bay, the Baltic and Mediterranean Seas (Grzebyk et al., 1996; Glibert et al., 2001; Fan et al., 2003; Heil et al., 2005). In recent years, there have been a number of studies on *P. minimum* in areas affected by freshwater runoff and/or anthropogenic inputs of nutrient, as well as on its association with protozoan grazers (Rosetta et al., 2003; Pertola et al., 2005; Peštová et al., 2008). It has been suggested that increased nutrient loading in near-shore waters may contribute to blooms of this dinoflagellate species and that ciliate may play a role in suppressing or shortening the duration of these harmful algal bloom (HAB) (Rosetta et al., 2003; Polat et al., 2007).

Semi-enclosed mariculture water-bodies are usually small-sized (<1 000 m^2), characterized by poor exchange of water with the sea, heavy disturbance from the introduction of cultured animals, and nutrient enrichment and/or contaminant inputs either from mariculture sources or of autochthonous origin (Pisani et al., 2008; Xu et al., 2008). This generally results in a eutrophic or hypertrophic environment, which is susceptible to recurrent eutrophication events and, thus, offers the opportunity

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to analyze the interactions between protists and a range of physico-chemical and biological parameters, especially population dynamics of HAB species and the relationship with ambient nutrients and grazers (Burford et al., 1998; 2003; Xu et al., 2008).

As a part of our investigations into the dynamics of planktonic marine protist communities, a 6-month baseline survey of the potential harmful dinoflagellate *P. minimum* was also carried out in a shrimp-farming pond near the coast of Qingdao, northern China. The present study focused mainly on: (1) the temporal population dynamics of *P. minimum,* and (2) the relationship with ambient nutrient inputs and protozoan grazers. The objective was to document the population dynamics of HAB species and their responses to suppressed and eutrophic conditions in a semi-enclosed mariculture water-body, and to provide statistical evidence that HABs may be suppressed or shortened by protozoan grazers.

2 MATERIALS AND METHODS

2.1 Study site, sampling, identification and enumeration

The study site was a small shrimp-farming pond located on the Laoshan Bay coast near Qingdao, China (Fig.1). During the period of study (May to October), the water depth in the pond was maintained at ~1.2 m by adding seawater from the sea every 2 weeks or so. Fifteen samples (designated 22-May, etc.) were collected every 10 days from May to October 2002. Sample treatment and identification/enumeration of planktonic protists followed the scheme of Xu et al. (2008). Analysis of inorganic parameters (DIN, sum of NO_3-N, NO_2-N and NH_3-N) and soluble reactive phosphate (SRP) followed standard methods (APHA, 1989).

2.2 Data analyses

All parameters were log-transformed prior to analyses. Data on salinity and temperature were available for all 15 samples. The relationship between abundance of *P. minimum* and environmental parameters were analyzed using Pearson's correlation matrix and multiple regression analysis. All analyses were carried using SPSS software version 16.0.

Because many of the variables were highly correlated, the contribution and significance of each environmental variable was first tested separately with regression analysis. The best possible multiple regression models were then explored using multiple
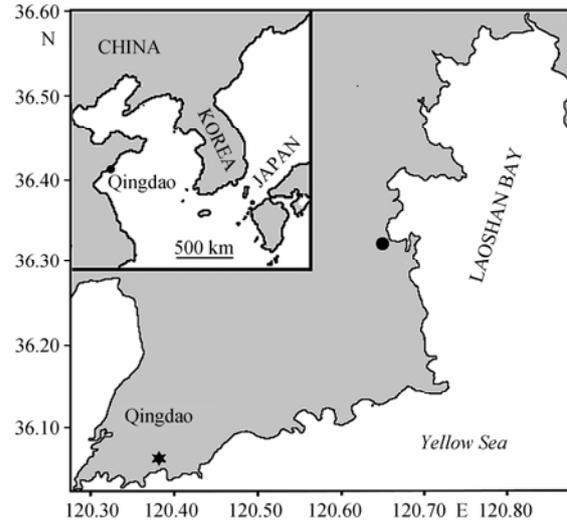

Fig.1 Map showing the location of the study site

linear regression. These were then explored using the stepwise selection mode of the SPSS software and the optimal model was estimated based on statistical significance (high R^2, $P<0.05$) and the biological relevance of the model parameters. The final results were calculated with the equation:

$$P_{m+1} = ax1^{b1}x2^{b2}\ldots xn^{bn} \qquad (1)$$

where P_{m+1} is the number of cells added by 1; a is the intercept, corrected with the mean-square error (through back-transformation of the estimated model); $x1^{b1}\ldots xn^{bn}$ are values for environmental or biotic parameters x and their parameter estimates b.

To clarify that regression results were dependent on the change in salinity and temperature and not on the absolute values of these parameters, regression models were also derived using the standardized values of salinity and temperature. Standardization followed the equation

$$u = \frac{x - x_0}{s} + d \qquad (2)$$

where u is the standardized value use in the model estimation; x is the absolute parameter value; x_0 is the mean of the absolute parameter values; s is the standard deviation of the absolute parameter values; d is a suitable constant value to make u a positive value for log-transformation. Accordingly, the mean of u was 0 and the standard deviation was 1. The final equation was:

$$P_{m+1} = a(x1 + d)^{b1}x2^{b2}\ldots xn^{bn} \qquad (3)$$

with symbols as in Eqs. 1 and 2.

3 RESULTS

3.1 Physico-chemical parameters

The values for seven environmental variables in each of the 15 samples are shown in Table 1. Water temperature ranged 19.5°C–31°C, leveling off steadily from May to June, increasing slowly and then dropping sharply after peaking in late August. Salinity averaged 28.7, maintaining high levels from May to the middle of July, but dropping sharply to the lowest level (8.1) in end of July (22-Jul sample) due to heavy rainfall, and reverting to its original levels from the end of July to the beginning of September. pH values remained relatively stable, ranging 7.24–8.05, while turbidities exhibited an increasing trend and peaked at the end of September. The average value of dissolved inorganic nitrogen (DIN, sum of NH_3-N, NO_2-N and NO_3-N) over the whole sampling period was 5.5 mg L^{-1}. There was an initial decline in DIN between May and mid-June, followed by an increasing trend after the introduction of shrimp juveniles. NH_3-N (mean 3.10 mg L^{-1}) represented 56% of total DIN and exhibited an increasing trend, whereas NO_3-N (mean 0.89 mg L^{-1}) and NO_2-N (mean 1.31 mg L^{-1}) leveled off steadily at a lower level. The concentrations of SRP ranged 0.01–6.24 mg L^{-1}, being much higher after the introduction of shrimp juveniles (13-June) than in pre-introduction period.

3.2 Temporal dynamics of *P. minimum* population

P. minimum was detected in almost all 15 samples from the semi-enclosed mariculture pond during the 6-month shrimp-farming cycle. There was a trend of increasing abundance, with a peak in October (Fig.2). Mean values were 3.6×10^4 cells L^{-1}, maintaining a low level from June to August, increasing from the middle of August to a maximum value of 2.2×10^5 cells L^{-1} (Fig.2). Likewise, the relative abundance of *P. minimum*, i.e., the percentage of phytoplanktonic protists, also showed an increasing pattern, with a mean value of 0.97% and two peaks in August (maximum value 6.87%) and in September (maximum value 1.85%) (Fig.2).

3.3 Correlation with physico-chemical parameters

Results obtained by multiple linear regression indicated that the abundance of *P. minimum* was correlated with physico-chemical factors (Table 2 and Fig.3A). However, none of the estimated optimal multiple regression models could explain the abundance of *P. minimum* cells. DIN was the most significant individual environmental variable relative to *P. minimum* abundance, explaining 37.6% of the variance in cell abundance (regression, $P<0.05$). In contrast, NO_x-N (NO_2-N + NO_3-N) and NO_2-N were the top two environmental parameters, explaining 40.7 and 39.9% of the variance, respectively (regression, $P<0.05$). The estimated models (Eq.1) are:

$$P_{m+1} = 7.534 \text{ DIN}^{3.883}$$

$$P_{m+1} = (3.597 \times 10^2) \text{ NO}_x\text{-N}^{3.687}$$

$$P_{m+1} = (2.286 \times 10^2) \text{ NO}_2\text{-N}^{4.200}$$

which demonstrated a positive relationship between cell abundance and nitrogen (Fig.3A).

Table 1 Environmental variables in mariculture pond water samples between May and October 2002

	T (°C)	S	pH	NH_3-N (mg L^{-1})	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	DIN (mg L^{-1})	SRP (mg L^{-1})
22-May	24.00	26.80	7.97	1.30	4.40	0.70	6.40	0.01
03-Jun	26.80	32.50	7.67	2.50	1.10	1.50	5.10	0.07
13-Jun	24.80	32.30	7.53	0.70	1.10	0.90	2.70	0.08
26-Jun	25.50	31.50	7.54	1.20	0.80	0.70	2.70	1.16
08-Jul	27.70	34.30	7.74	2.00	1.00	1.10	4.10	6.48
18-Jul	27.30	32.70	7.79	2.50	0.90	0.60	4.00	4.34
26-Jul	27.70	8.30	7.87	3.60	0.90	0.60	5.10	4.80
05-Aug	27.70	21.70	8.05	3.50	1.30	1.20	6.00	5.92
15-Aug	29.10	25.10	7.58	1.80	0.90	1.00	3.70	1.86
25-Aug	31.00	28.50	7.87	2.60	0.80	1.20	4.60	5.10
04-Sep	27.70	31.30	7.84	5.50	1.30	1.20	8.00	5.72
14-Sep	21.00	29.20	7.59	1.60	1.10	0.90	3.60	4.56
24-Sep	23.00	32.50	7.75	3.90	1.80	1.20	6.90	6.24
04-Oct	23.00	32.30	8.01	5.90	2.40	1.10	9.40	5.60
14-Oct	19.50	32.10	7.95	4.30	2.10	1.30	7.70	5.20

DIN: sum of NH_3-N, NO_2-N and NO_3-N; S: salinity; SRP: soluble reactive phosphate; T: temperature
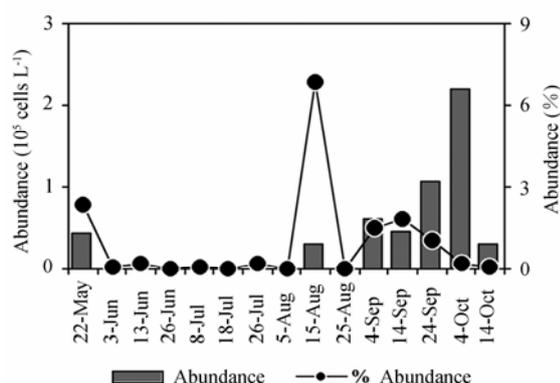
Fig.2 Temporal variations in *Prorocentrum minimum* in absolute and relative abundance (%) from May to October 2002

The estimated regression model with standardized temperature verified that temperature change was the significant factor in relation to *P. minimum* abundance, while absolute parameter values were not as important. The estimated model (Eq. 2)

$$P_{m+1} = (1.244 \times 10^5)(\text{temp} + 1.979)^{-2.655}$$

explained 29.7% of *P. minimum* abundance ($P<0.001$), revealing a negative correlation between cell abundance and standardized values of temperature.

3.4 Correlation with protozoan grazers

During the study period, *P. minimum* co-occurred with the four planktonic ciliates (*Gyrodinium spirale*, *Mesodinium pupula*, *Strobilidium neptuni*, *Tintinnopsis lohmanni*, *Askenasia stellaris*), and one heterotrophic dinoflagellate, *Gyrodinium spirale* (Fig.3B). Statistical analysis showed that *P. minimum* was significantly correlated with these protozoan grazers (Table 3). The optimal multiple regression model (Eq. 1)

$$P_{m+1} = 1.416 \ M.\ pupula^{0.733}S.\ neptuni^{4.145}T.\ lohmanni^{-0.810}A.\ Stellaris^{-1.001}$$

explained 97.9% of its abundance with an increase in *Mesodinium pupula* and *Strobilidium neptuni*, and a decrease of *Tintinnopsis lohmanni* and *Askenasia stellaris*.

4 DISCUSSION

In this study, the temporal population dynamics of *P. minimum* supported previous evidence that this species occurs in higher number mainly during late summer or early autumn, and appears to benefit from nutrient input (EPA, 2003; Pertola et al., 2005). However, cell numbers were much lower than the common bloom threshold value (3×10^6 cells L^{-1}) reported for the Baltic Sea, Chesapeake Bay and a shrimp-farming pond near Sinaloa, Mexico (EPA, 2003; Pertola et al, 2005; Tango et al., 2005; Sierra-Beltrán et al., 2005).

The abundance of *P. minimum* also presented a strong positive correlation with nitrogen and a

Table 2 Linear regression analysis (abundance of *Prorocentrum minimum* relative to physico-chemical parameters)

Parameters	R^2	Variables	Regression coefficients	T	P
DIN	0.376	Constant	-2.123	-1.931	0.076
		Variable	3.883	2.799	0.015
NO$_2$-N+ NO$_3$-N	0.407	Constant	-0.444	-0.909	0.380
		Variable	3.687	2.985	0.011
NO$_2$-N	0.399	Constant	-0.641	-1.147	0.272
		Variable	4.200	2.936	0.012
Water temperature	0.297	Constant	2.095	3.862	0.002
		Variable	-2.655	-2.341	0.036

Table 3 Linear regression analysis (abundance of *Prorocentrum minimum* related to algivorous protists (F of the model is only shown when the model is multiple))

Species	R^2	Variables	Regression coefficients	T	P
Mesodinium pupula	0.469	Constant	0.480	2.255	0.042
		Variable	0.639	3.387	0.005
Strobilidium neptuni	0.458	Constant	0.619	3.205	0.007
		Variable	2.995	3.318	0.006
Gyrodinium spirale	0.402	Constant	0.329	1.234	0.239
		Variable	0.532	2.959	0.011
Mesodinium pupula	0.979	Constant	0.151	2.860	0.017
Strobilidium neptuni		Variable 1	0.733	13.910	<0.001
Tintinnopsis lohmanni		Variable 2	4.145	14.301	<0.001
Askenasia stellaris		Variable 3	-0.810	-7.098	<0.001
$F_{(4,14)}$ model: 116.118; $P<0.001$		Variable 4	-1.001	-2.535	0.030
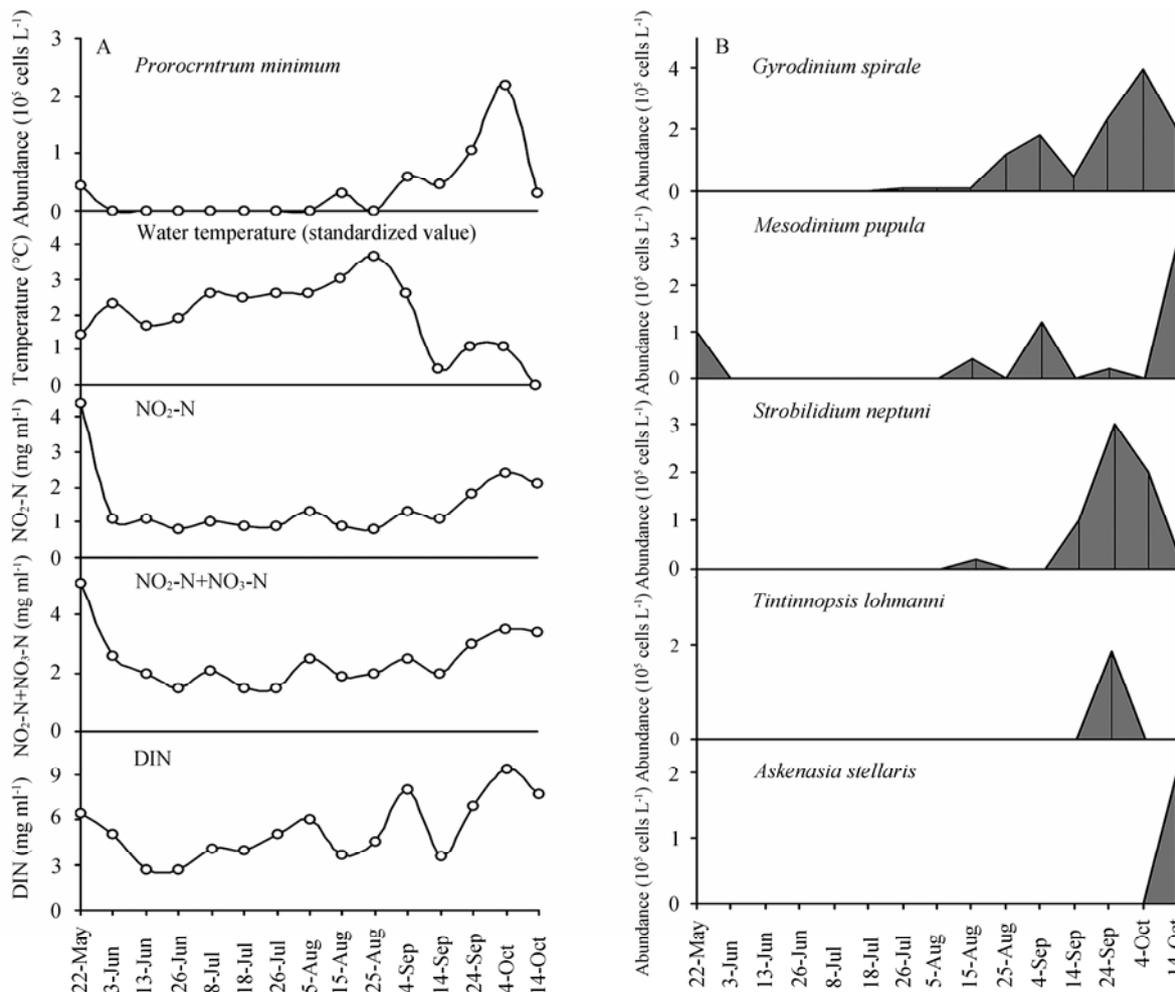
Fig.3 Abundance dynamics of *Prorocentrum minimum* in relation to (A) environmental parameters (water temperature and NO₂-N, NO₂-N+NO₃-N, DIN) and (B) various algivorous protozooplankton species: a heterotrophic dinoflagellate, *Gyrodinium spirale,* and four ciliates, *Mesodinium pupula*, *Strobilidinium neptuni*, *Tintinnopsis lohmanni* and *Askenasia stellaris*

negative correlation with temperature. These results are consistent with previous findings-the correlation between *P. minimum* abundance and total nitrogen; the inverse relationship between *P. minimum* and temperature, representing a seasonal progression from August to October; and the capability of *P. minimum* to grow in relatively low temperatures (optimal values 12°C–28°C) in the Baltic Sea and Chesapeake Bay (Pertola et al., 2005; Tango et al., 2005).

During the study period, the concentration of input nutrients showed an increasing trend, resulting in eutrophic or hypertrophic conditions that promote microalgal blooms. This is in agreement with the positive relationship between cells and DIN data, and a previous suggestion that *P. minimum* attains a competitive advantage under relatively cool, turbid and nutrient-enriched conditions, including the thermophilic clones, which have optimal growth

between 18 and 26.5°C (Grzebyk et al., 1996; Smayda et al., 2001; Burford et al., 2003).

P. minimum appears to be a good competitor when nitrate is exhausted from the water during the early phase of the bloom and high concentrations of total nitrogen are available (Pertola et al., 2005). In this study, however, nitrite+nitrate was positively related to cell numbers. This may be due to the fact that nitrogen is not the common limiting nutrient factor in a semi-enclosed mariculture pond.

Regarding the estimated optimal multiple regression model, in the Baltic Sea and Chesapeake Bay, *P. minimum* abundance can be explained by decreased salinity — in the optimal range 4.5–12.8 (Pertola et al., 2005; Tango et al., 2005). In the present study, however, this correlation was not evident, almost certainly due to high salinity. Experimental studies have shown that *P. minimum* can grow over a broad salinity range (Heil et al.,

2005). Hajdu et al. (2000) reported growth of the Baltic clones between 2–35, but optimal growth occurred at 15–17. In our study, salinity (28.7 ± 6.6) was clearly outside the optimal range for *P. minimum* growth over the whole farming cycle. This may also be the reason why *P. minimum* did not dominate the phytoplankton community, i.e., its relative abundance was maintained at low levels.

It has been suggested that top-down control by zooplankton grazers may prevent bloom formation or shorten the duration of HABs. The specific role of microzooplankton in grazing harmful or nuisance phytoplankton is poorly understood (Banse, 1994; Buskey et al., 1997; Turner et al., 1997). Several studies have reported that the density of protozoan grazers in algal blooms is negatively related to the loss of phytoplankton biomass. For example, the planktonic ciliate *Favella ehrenbergii* has been credited with grazing down dinoflagellate blooms, while other ciliates have been reported to attain optimal reproduction and growth rates when fed some HAB species (Stoecker et al., 1981; Jeong et al., 1999). With reference to the relationship between *P. minimum* and protozoan grazers, Rosetta et al. (2003) suggested that prior to the onset of toxicity and bloom formation, ciliate grazers may exert grazing pressure on HAB species, potentially contributing to the suppression or decline of *P. minimum* and *P. parvum* blooms.

In the present study, multiple regression analysis revealed that *P. minimum* numbers showed a significant inverse relationship to the abundance of the planktonic ciliates, *Tintinnopsis lohmanni* and *Askenasia stellaris*. This finding agrees with the reports mentioned above and, thus, provides statistical evidence that *P. minimum* bloom may be suppressed or shortened by protozoan grazers under the toxicity threshold before bloom formation in semi-enclosed mariculture water bodies.

In conclusion, our results demonstrate that *P. minimum* occurred frequently in low numbers in a semi-enclosed mariculture pond near Qingdao and that its temporal population pattern was positively correlated with nutrient nitrogen but inversely related to the planktonic ciliates, *Tintinnopsis lohmanni* and *Askenasia stellaris*. This provides statistical evidence that *P. minimum* may become dominant with increasing nitrogen levels, and suppression or shortening of the bloom may be associated with protozoan grazers, such as *Tintinnopsis lohmanni,* in mariculture water bodies. To verify this conclusion, however, further investigations are required on a size-range of semi-enclosed mariculture ponds over extended time periods.

5 ACKNOWLEDGEMENTS

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