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Regulation of phosphate uptake kinetics in the bloom-forming dinoflagellates *prorocentrum donghaiense* with emphasis on two-stage dynamic process

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ABSTRACT

Phosphorus is an essential element for the growth and reproduction of algae. In recent years, the frequent outbreaks of algal blooms caused by eutrophication have drawn much attention to the influence of phosphate (P) uptake on the growth of algal cells. The previous study only considered the effect of total P pools on the P uptake process of algal cells and considered the process as one stage, which is insufficient. P uptake by algae is actually a two-stage kinetic process because in many algae species, surface-adsorbed P pools account for a large proportion of total P pools. In this paper, we fit one-stage and two-stage models of P uptake by algae to our experimental data on short-term uptake kinetics of algae *Prorocentrum donghaiense* under P-deplete and P-replete conditions at 24°C. According to the experimental results, *P. donghaiense* possesses different P uptake characteristics under different P concentrations. *P. donghaiense* grows faster and exponentially for longer periods of time under P-replete condition. Ranges of change of Q_c (cell quota of intracellular P) and S_p (cell quota of surface-adsorbed P) during the culture time are obviously larger under P-replete condition than those under P-deplete condition. The value of K_p (represents the impact of P-starvation on P uptake rate) in one-stage model under P-deplete condition is smaller than that under P-replete condition, which is opposite to results of two-stage model and does not meet the actual biological significance of K_p. The two-stage model gives more reasonable and realistic explanations to the process of P uptake by algae no matter from the perspective of intuitive fitting effect, biological significance of parameters, statistical test results or essential dynamic process. These results, combined with long-term lab and field data in ocean, could be used to effectively predict algal blooms.

1. Introduction

Harmful algal blooms (HABs) are an increasingly serious marine environmental problem for aquaculture, fisheries and public health in many coastal areas throughout the world (Anderson, 1997), because recent research findings show that in many regions, harmful phytoplankton blooms are occurring more frequently and lasting longer (Zhao et al., 2016; Yu et al., 2018). HABs have become common in the Yangtze River estuary and the East China Sea (ECS) since the 1980s and the frequency of dinoflagellate blooms appears to have increased since 2000 (Zhao, 2010). *Prorocentrum donghaiense* (formerly named *P. dentatum*; Gómez, 2005; Lu et al., 2005) has been a major bloom-forming dinoflagellates species in the Yangtze River estuary and the adjacent coastal waters of the ECS during the annual blooms that occur in late April and May since 2000 (Tang et al., 2006; Zhao, 2010; Shen et al., 2016). Nutrients are important environmental factors that are related to microalgae growth and can affect the population dynamics of individual species and the species succession in the field (Karentz and Smayda, 1984). The species that is able to successfully compete for the available growth-limiting nutrient has the potential to become dominant by increasing its biomass and ultimately form a bloom (Granéli and Hansen, 2006). In addition, the phosphate (P) concentration in the coastal water of the ECS is low after diatoms blooms (Li et al., 2009; Zhao, 2010). Therefore, it is necessary to understand the relationship between P uptake and phytoplankton *P. donghaiense* growth under different P conditions.

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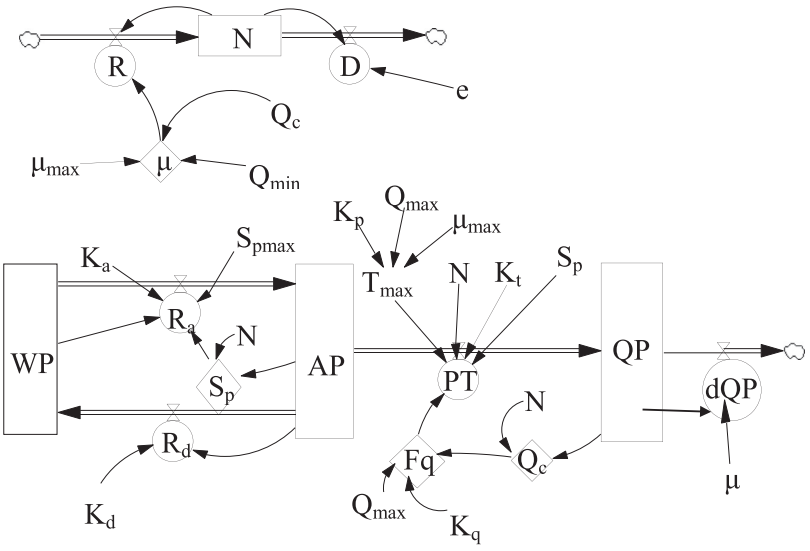

Fig. 1. The diagram of the two-stage model. Boxes are levels, circles are rates, diamonds are auxiliaries, square brackets are shadow variables, clouds represent sources or sinks, and the others are constants.

Over the past few decades, the transport dynamics into cells have been confirmed in nutrient uptake experiments of many phytoplankton. Correspondingly, the uptake kinetics of algae are always described by Michaelis–Menten equation where the parameters V_{max} (maximum uptake rate) and K_s (half-saturation constant) are estimated based on experimental data and could be used to interpret the characteristics of different species of microalgae (Aksnes and Egge, 1991). In the beginning, this kind of method could describe the uptake process efficiently. However, the extensive use of Michaelis–Menten kinetics to describe nutrient uptake appears to conceal the difficulties resulted from the interpretation of kinetic parameters based on measurements (Droop, 1983). Therefore, it is insufficient to only use Michaelis–Menten kinetics to describe nutrient uptake process of microalgae.

Nutrient concentration of substrate is not considered by Michaelis–Menten kinetics (Aksnes and Egge, 1991). Nutrient uptake of microalgae could increase due to nutrient deficiency (Syrett, 1956) and maximum short-term uptake rate reasonably depends on the previous level of nutritional stress (Morel, 1987). The results of some experiments demonstrate that cells with P-stress have higher P uptake rates than cells without P-stress (Goldman and Glibert, 1982; Lehman and Sangren, 1982; Riegman and Mur, 1984; Lin et al., 2016). Algae absorb P in the medium very quickly in P-deplete condition. It may uptake P by 8–16 times of the minimum cell-quota in P-replete medium and accumulate P for sustaining the growth of 3–4 generations in the P-deplete medium (Droop, 1973; Morel, 1987). These studies do not support the 2-parameter Michaelis–Menten model. Therefore, Harrison et al. (1989) suggested that the maximum uptake should be defined both for cells with replete and deplete P supply and the uptake rate should be related to the size of the intracellular P pool.

In addition to intracellular P pool size (directly related to environmental P concentration), surface-adsorbed P pool is also of great significance on the P uptake of algae. Almost all previous studies only consider the transportation from substrate into cells, which shows P uptake as only a one-stage dynamic process. However, a few studies (Sãnudo-Wilhelmy et al., 2004) suggest that surface adsorption matters and the process should be two-stage. Why does surface-adsorbed P matter? Firstly, in different species of algae, the surface-adsorbed P may account for 15% to 45% of total cellular P (Tovar-Sanchez et al., 2003; Sãnudo-Wilhelmy et al., 2004; Fu et al., 2005; Saxton et al., 2012). Obviously, the coexistence of surface-adsorbed and intracellular P pool indicates that two stages are needed to describe the P uptake process in algae: (1) adsorption and desorption between the substrate and the cell surface; (2) transportation from surface-adsorbed P into cells. Secondly, the adsorption and desorption at the substrate-cell interface is an instantaneous process. Changes of environmental conditions such as P concentration, temperature, hydrodynamic environment, pH of the substrate may change the direction of the P flux between the substrate and the cell surface (Harrison et al., 1989; Aksnes and Egge, 1991). Then the concentration of surface-adsorbed P could be changed, which affects the size of intracellular P pool eventually. Furthermore, cell growth directly depends on the intracellular P, rather than surface-adsorbed P (Droop, 1973). Separating total cellular P into surface-adsorbed and intracellular P pool is beneficial to describe the rule of cell growth. Therefore, it is extremely necessary to consider surface-adsorbed P pool in the P uptake kinetics research.

What kind of model that could interpret the P uptake kinetics in algae most accurately? To answer this question, based on the conventional one-stage model, we have formulated a two-stage model of P uptake by algae incorporating both surface adsorption and P concentration in substrate. Our model incorporates P adsorption/desorption between the substrate and cell surface, intracellular transportation of surface-adsorbed P into cells, and cell growth with respect to intracellular P quota. To verify the rationality of our model, we perform experiments on *P. donghaiense* and estimate the parameter values in our model based on experimental data of Q_t (cell quota of intracellular P plus surface-adsorbed P) and QP/(AP + QP) (proportion of intracellular P in total P of cells). Furthermore, we discuss the P uptake characteristics of bloom-forming dinoflagellates *P. donghaiense* under different conditions (environmental P concentration), compare one-stage (conventional) model with our two-stage model and analyze shortcomings and advantages of two models. Surface-adsorbed P may affect P uptake processes and assist in understanding realistic P uptake kinetics in phytoplankton, and environmental P concentration may play pivotal roles in P uptake processes.

Table 1
Notations, units and explanations of state variables on one-stage and two-stage models.

Variable	Unit	Explanation
WP	μM	P concentration in substrate
AP	μM	concentration of surface-adsorbed P
QP	μM	concentration of intracellular P
N	10^8 cells L^{-1}	cell density of algae
TP	μM	The sum of AP and QP

TP only used in one-stage model.

2. Materials and methods

2.1. Model description of two-stage model

We formulate our two-stage model based on Yao et al. (2011) except changing the specific growth rate into the classic Droop equation (Riegman and Mur, 1984). P uptake process by algae includes two steps: ((1) substrate P (WP) is transported to algal cell surfaces. (2) surface-adsorbed P (AP) is transported into the cells through the cell membrane and then becomes intracellular P (QP). We only consider dissolved inorganic P (DIP) and the model is formulated under the assumption that dissolved organic P (DOP) is unable to be converted into DIP after the death of algal cells. Therefore, total amount of P (WP + AP + QP) is not constant over time. Variables and parameters needed by the two-stage model or one-stage (conventional) model are provided in Tables 1 and 2 respectively. S_p represents the cell quota of surface-adsorbed P, and Q_c represents the cell quota of intracellular P. Mathematically, S_p and Q_c equals AP/N and QP/N respectively. A mathematical model that describes P uptake kinetics on algae (two-stage model) is given by (1) and Fig. 1 is the diagram of the model. The biological meanings and notations of these functions and formula are shown below the brackets in (1), which are consistent with the notations in Fig. 1.

$$\begin{cases} \frac{dN}{dt} = \underbrace{\underbrace{\mu_{\max}\left(1-\frac{Q_{\min}}{Q_c}\right)}_{\mu:Droop\ equation} N}_{R:cell\ growth} - \underbrace{eN}_{D:cell\ death}, \\ \frac{dAP}{dt} = \underbrace{K_a WP\left(1-\frac{S_p}{S_{p\max}}\right)}_{R_a:absorption\ from\ substrate} - \underbrace{K_d AP}_{R_d:desorption} \\ \qquad - \underbrace{\underbrace{K_p Q_{\max}\mu_{\max}\frac{S_p}{S_p+K_t}\frac{\left(1-\frac{Q_c}{Q_{\max}}\right)^4}{\left(1-\frac{Q_c}{Q_{\max}}\right)^4+K_q} N}_{F_q:feedback\ function}}_{PT:P\ transport\ rate}, \\ \frac{dQP}{dt} = k_p Q_{\max}\mu_{\max}\frac{S_p}{S_p+K_t}\frac{\left(1-\frac{Q_c}{Q_{\max}}\right)^4}{\left(1-\frac{Q_c}{Q_{\max}}\right)^4+K_q} N - \underbrace{\mu_{\max}\left(1-\frac{Q_{\min}}{Q_c}\right)QP}_{dQP:dilution\ due\ to\ growth\ and\ birth}, \\ \frac{dWP}{dt} = -K_a WP\left(1-\frac{S_p}{S_{p\max}}\right) + K_d AP. \end{cases} \tag{1}$$

Here, phosphate transport is also controlled by the feedback of internal P pool size and can be described by a sigmoidal function with a power of 4 (Flynn et al., 1997; John and Flynn, 2000; Flynn, 2003):

$$\frac{\left(1-\frac{Q_c}{Q_{\max}}\right)^4}{\left(1-\frac{Q_c}{Q_{\max}}\right)^4+K_q}.$$

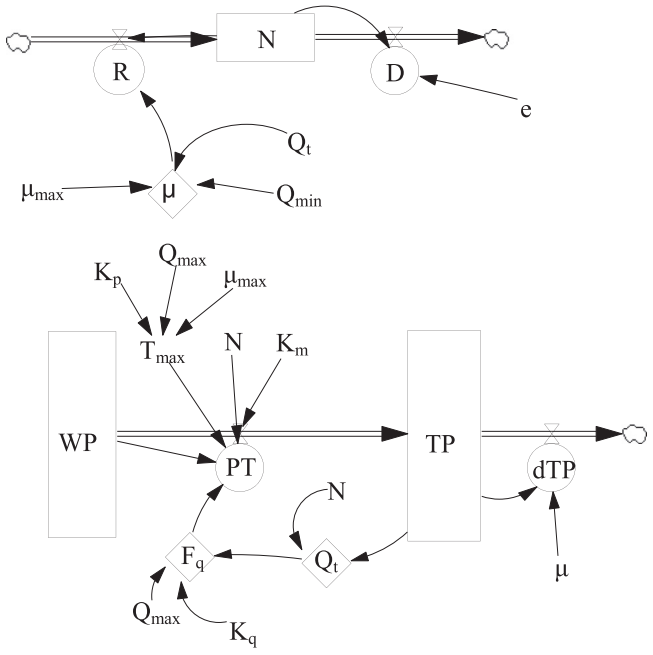

Fig. 2. The diagram of the conventional one-stage model.

2.2. Model description of conventional one-stage model

Conventional model does not take the effect of surface-adsorbed P into consideration. Therefore, in a conventional one-stage model, Q_t represents the cell quota of intracellular P plus surface-adsorbed P, not only the cell quota of intracellular P. TP represents concentration of surface-adsorbed P plus intracellular P, not only concentration of intracellular P. Therefore, Q_t equals TP/N mathematically. Especially, the form of P transport rate per algal cell could be described as

$$K_p Q_{t\max}\mu_{\max}\frac{WP}{WP+K_m}\underbrace{\frac{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4}{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4+K_q}}_{F_q:feedback\ function}, \tag{2}$$

where K_m (μM) is the half-saturation constant for the substrate concentration at which the P transport rate attains half of its maximum. A conventional one-stage model that describes P uptake kinetics on algae is given by (3) and Fig. 2 is the diagram of the model. The biological meanings and notations of these functions and formula are shown below the brackets in (3), which are consistent with the notations in Fig. 2.

$$\begin{cases} \frac{dN}{dt} = \underbrace{\underbrace{\mu_{\max}\left(1-\frac{Q_{t\min}}{Q_t}\right)}_{\mu:Droop\ equation} N}_{R:cellgrowth} - \underbrace{eN}_{D:cell\ death}, \\ \frac{dTP}{dt} = \underbrace{\underbrace{K_p Q_{t\max}\mu_{\max}\frac{WP}{WP+K_m}\frac{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4}{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4+K_q} N}_{F_q:feedback\ function}}_{PT:P\ transport\ rate} \\ \qquad - \underbrace{\mu_{\max}\left(1-\frac{Q_{t\min}}{Q_t}\right)TP}_{dQP:dilution\ due\ to\ growth\ and\ birth}, \\ \frac{dWP}{dt} = -K_p Q_{t\max}\mu_{\max}\frac{WP}{WP+K_m}\frac{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4}{\left(1-\frac{Q_t}{Q_{t\max}}\right)^4+K_q} N. \end{cases} \tag{3}$$

Table 2
Notations, units and explanations of parameters on one-stage and two-stage models.

Parameter	Unit	Explanation
K_p	constant	coefficient to reflect P-starvation
e	day^{-1}	death rate of algae
K_a	day^{-1}	adsorption constant
K_d	day^{-1}	desorption constant
K_q	constant	used to control the feedback function
K_t	10^{-8} μ mol $cell^{-1}$	half-saturation constant for two-stage model
μ_{max}	day^{-1}	maximum growth rate of algae
Q_{max}	10^{-8} μ mol $cell^{-1}$	maximum cell quota of intracellular P
S_{pmax}	10^{-8} μ mol $cell^{-1}$	maximum cell quota of surface-adsorbed P
Q_{min}	10^{-8} μ mol $cell^{-1}$	minimum cell quota of intracellular P
Q_{tmax}	10^{-8} μ mol $cell^{-1}$	maximum cell quota of intracellular P plus surface-adsorbed P
Q_{tmin}	10^{-8} μ mol $cell^{-1}$	minimum cell quota of intracellular P plus surface-adsorbed P
K_m	μM	half-saturation constant for one-stage model

Q_{tmax}, Q_{tmin}, K_m only used in one-stage model.

Table 3
Sensitivity analysis based on two-stage model.

Parameter	Control value	Test value	Model response			
			WP	AP	QP	N
K_p	2	1	2.05	0.072	1.4	5.3
		4	5.70	0.6	3.8	1
e	0.037	0.018	0.19	0.03	0.3	0.1
		0.074	0.44	0.08	0.524	0.170
K_a	0.072	0.036	1.23	0.7	0.7	0.207
		0.145	2.23	2.063	0.45	0.2
K_d	1.44	0.72	1.61	0.7	0.6	0.15
		2.88	1.5	0.6	0.3	0.163
K_q	127.43	63.72	5.6	1	3	0.96
		254.86	3	0.385	0.6	9.253
K_t	0.63	0.32	0.9	0.168	0.8	0.3
		1.27	0.2	0.2	0.2	0.02
μ_{max}	0.38	0.19	3.25	0.4	2	0.6
		0.76	9.8	0.738	1.5	900
Q_{max}	198.34	99.17	0.17	0.1	1.4	8.5
		396.68	6	0.3	3	1
S_{pmax}	33.68	16.84	0.3	0.04	0.1	0.1
		67.36	0.2	0.02	0.034	0.012
Q_{min}	3.07	1.54	3.4	0.78	0.9	1.5
		6.15	3.9	0.4	0.5	8.8

Table 4
Sensitivity analysis based on one-stage model.

Parameter	Control value	Test value	Model response		
			WP	TP	N
K_p	2	1	5	2.71	0.05
		4	0.3	1.84	0.03
e	0.154	0.077	0.3	1.96	0.33
		0.308	3	2.63	0.3
K_q	1.779×10^{-10}	8.893×10^{-11}	4.0×10^{-5}	6×10^{-5}	0.1
		3.557×10^{-10}	4.2×10^{-5}	6×10^{-5}	0
μ_{max}	2.46	1.23	11	1.3	0.3
		4.91	0.3	2.10	0.04
Q_{tmax}	52.00	26.00	13	1.4	0.2
		104.00	0.3	2	0.07
Q_{tmin}	8.40	4.20	0.113	0.8	0.11
		16.80	0.4	1.3	0.22
K_m	231.91	115.95	0.3	1.75	0.06
		463.82	4.5	2	0.03

2.3. Sensitivity analysis

To understand how system parameters influence state variations (Roelke et al., 1999), we carry out local sensitivity analysis for all parameters in two-stage model and conventional model with the initial values of the state variables WP, AP, QP and TP as 35.22 μM, 1.87 μM, 1.49 μM, 3.36 μM and the initial values of N as 0.15×10^8 cells L^{-1}.

We run the two-stage model and the conventional model separately with the control value, double or half of the control value of those parameters. For each run, one of the parameters is varied by a factor of either 0.5 or 2.

The model response is calculated as the absolute value of $(P_a - P_c)$, where P_a and P_c refer to values of state variables after 12 days using the altered value (double or half of the control value) and the control values of those parameters, respectively. The results of the sensitivity analysis are given in Tables 3 and 4, which suggest that there is no particularly sensitive parameter on both two-stage model and conventional model. Therefore, the quantitative results are robust.

In two-stage model, WP is mainly affected by K_p, as it quantizes the level of P-stress and it is also heavily determined by μ_{max}, Q_{max}, Q_{min} because those parameters decide the level of P concentration in substrate and specific maximum growth rate of algae. AP is mainly affected by K_a, since K_a represents surface adsorption characteristics. K_p, K_q and Q_{max} influence QP mostly as these parameters control the demand of intracellular P and P transport rate (see (1)). Algal cell density (N) is significantly affected by μ_{max} and N is also affected by K_p, K_q, Q_{max}, Q_{min}.

In one-stage model, Q_{tmax}, K_m, μ_{max}, K_p affect WP mostly since those parameters regulate the amount and speed of P absorption by algal cells from substrate into cells. Parameters e and μ_{max} affect TP and N directly.

2.4. Experimental materials and methods

2.4.1. Algal species and culture conditions

Prorocentrum donghaiense was provided by Prof. Lu of the Second Institute of Oceanography, State Oceanic Administration of the People's Republic of China (SOA) in Hangzhou, China. The algae was grown at 24 °C in f/2 medium (Guillard, 1975), which contained autoclaved (121 °C, 20 min) seawater. The cultures were maintained at a light intensity of 65–70 μ mol photons m^{-2} s^{-1} under a 12:12 h light: dark cycle in illuminating incubators (MTI-201B, Rikakikai, Japan). All cultures were manually shaken twice daily at a set time. The microalgae were cultivated to the exponential growth phase for use.

2.4.2. Phosphate uptake experiments

The cultures included 30 ml of a 12-day old culture (the cell density of about 1.0×10^5 cells mL^{-1}) and 170 ml fresh f/2 (P-replete group, 35.21 μM P) or fresh f/2 excluding $NaH_2PO_4 \bullet H_2O$ (P-deplete group, 2.88 μM P) enriched seawater to give a cell density

of about 1.5×10^4 cells mL^{-1}. The P uptake experiments were carried with three biological repeats. During the experiment, 10 mL of culture was sampled every 3 days (*i.e.*, day 0, day 3, day 6, day 9, and day 12). The detection methods of WP, AP, and QP according to Yao et al. (2011) with minor modification, (1) Each sample was filtered by 0.22 μm cellulose acetate membrane immediately after sampling, and P concentration in the filtrate was analyzed with phosphomolybdate-blue spectrophotometry for WP; and (2) the filtration membrane with microalgal cells was then carefully washed by 10 mL oxalic acid reagent and filtered again by 0.22 μm cellulose acetate membrane, in which the filtrate was analyzed with phosphomolybdate-blue spectrophotometry for AP, and microalgal cells on the filtered membrane were carefully washed out by 10 mL MQ water, digested by potassium persulfate method, and analyzed with phosphomolybdate-blue spectrophotometry for QP. A 0.5 ml sample was collected and preserved in Lugol's solution to monitor the growth of the microalgae by directly counting the cell density (N), 0.1 ml samples were counted in a phytoplankton counter frame (CC-F, Beijing purity instrument, Co., Ltd., China) with an optical microscope (BX43, Olympus, Japan) at day 0, day 3, day 6, day 9, and day 12, respectively.

3. Results

3.1. Experimental results

Fig. 3 shows the state variables N, WP, cell quota of intracellular P (Q_c), cell quota of surface-adsorbed P (S_p) of *P. donghaiense* under P-replete and P-deplete conditions at 24 °C. The results of *P. donghaiense* under P-replete condition are described below. With initial cell density 0.15×10^8 cells L^{-1}, N decreased slowly during treatment time of 3 days and increased rapidly in later days (Fig. 3a). With the initial P concentration in substrate 35.2 μM, WP decreased until the end of the experiment (Fig. 3b). With the initial Q_c 9.94×10^{-8} μ mol $cell^{-1}$, Q_c showed a periodic oscillation with 6 days as a cycle, but the amplitude became smaller and smaller as time elapses (Fig. 3c). With the initial S_p 12.49×10^{-8} μ mol $cell^{-1}$, S_p grew during treatment time of 3 days and declined in the next 9 days (Fig. 3d).

The results of *P. donghaiense* under P-deplete condition are described below. With initial cell density 0.15×10^8 cells L^{-1}, N increased during treatment time of 9 days and decreased in later 3 days (Fig. 3a). With the initial WP 2.87 μM, WP declined slowly during the whole treatment time (Fig. 3b). With the initial Q_c 11.02×10^{-8} μ mol $cell^{-1}$, Q_c began to decline after the first three days of increase and began to rebound in the last three days (Fig. 3c). With the initial S_p 11.05×10^{-8} μ mol $cell^{-1}$, S_p increased slowly in the first three days and declined gradually from day 3 to day 9, showing a slow decline in the last three days (Fig. 3d). According to these results, P concentration in substrate influences the growth of *P. donghaiense* significantly.

3.2. Fitting of the experimental data

3.2.1. Parameters used to fit the experimental data

The two-stage and one-stage P uptake models described above are implemented by system dynamic software VENSIM® (Vensim DSS32, Version 5.4a). The values of parameters on two-stage P uptake model are estimated based on experimental data QP/(QP + AP) of *P. donghaiense* and the values of parameters on one-stage P uptake model are estimated based on experimental data Q_t of *P. donghaiense* by using four-order Runge–Kutta method. Since there are too many parameters (10 and 7) in one-stage model and two-stage model, we set some parameters that express basic characteristics as constants, whose values are calculated and assumed based on previous studies (John and Flynn, 2000; Yao et al., 2011; Saxton et al., 2012). These results are listed in Tables 5–7. From these tables, values of K_p on two-stage model under P-deplete condition are larger than those under P-replete condition, which is opposite to results of one-stage model.

Table 5
Parameter values of two-stage model based on experimental data of QP/(QP + AP) in *P. donghaiense*.

Parameter	P-replete	P-deplete
K_p	1.00	3.00
e	0.003	0.3
μ_{max}	0.375	0.389
K_a	0.05	4.51
K_d	1	0.9
K_q	129.10	15
K_t	2	70.34

Table 6
Parameter values of one-stage model based on experimental data of Q_t in *P. donghaiense*.

Parameter	P-replete	P-deplete
K_p	1.86	0.122
μ_{max}	0.6	1.11
e	0.0001	0.1
K_q	0.037	1.8×10^{-10}
K_m	0.00029	0.00066

Table 7
Parameters set as constant during the fitting process and their values.

Parameter	Unit	value
Q_{max}	10^{-8} μ mol $cell^{-1}$	180
S_{pmax}	10^{-8} μ mol $cell^{-1}$	33
Q_{min}	10^{-8} μ mol $cell^{-1}$	6.7
Q_{tmax}	10^{-8} μ mol $cell^{-1}$	52
Q_{tmin}	10^{-8} μ mol $cell^{-1}$	5.2

3.2.2. Experimental data and fitted curve of q_t and QP/ (AP + QP)

Fig. 4 shows experimental data and fitted curve for Q_t of one-stage model in *P. donghaiense* using parameter values given above. Q_t in *P. donghaiense* increases first and then decreases, which is not affected by P concentration in substrate. One-stage model under P-replete condition cannot fit the experimental data for Q_t in *P. donghaiense* as well as that under P-deplete condition.

Fig. 5 shows experimental data and fitted curve for QP/(AP + QP) of two-stage model in *P. donghaiense* using parameter values given above. QP/ (AP + QP) in *P. donghaiense* increases soon after it attains its minimum, which is a common phenomenon regardless of P concentration in substrate. From Fig. 5, two-stage and one-stage models fit the experimental data for QP/ (AP + QP) in *P. donghaiense* well.

4. Discussion

In this paper, through the study of the P uptake kinetics of the common red tide dinoflagellates *P. donghaiense*, we find that *P. donghaiense* has different uptake characteristics under different P concentrations. In addition, in view of the fact that dinoflagellate cells have surface-adsorbed and intracellular P pools, the two-stage kinetic model can better describe the transport status of P in water bodies, cell surfaces and internal cells.

Phosphorus is the main element necessary for the growth of phytoplankton. Phosphorus is directly involved in all aspects of the photosynthesis process, including light energy absorption elec-

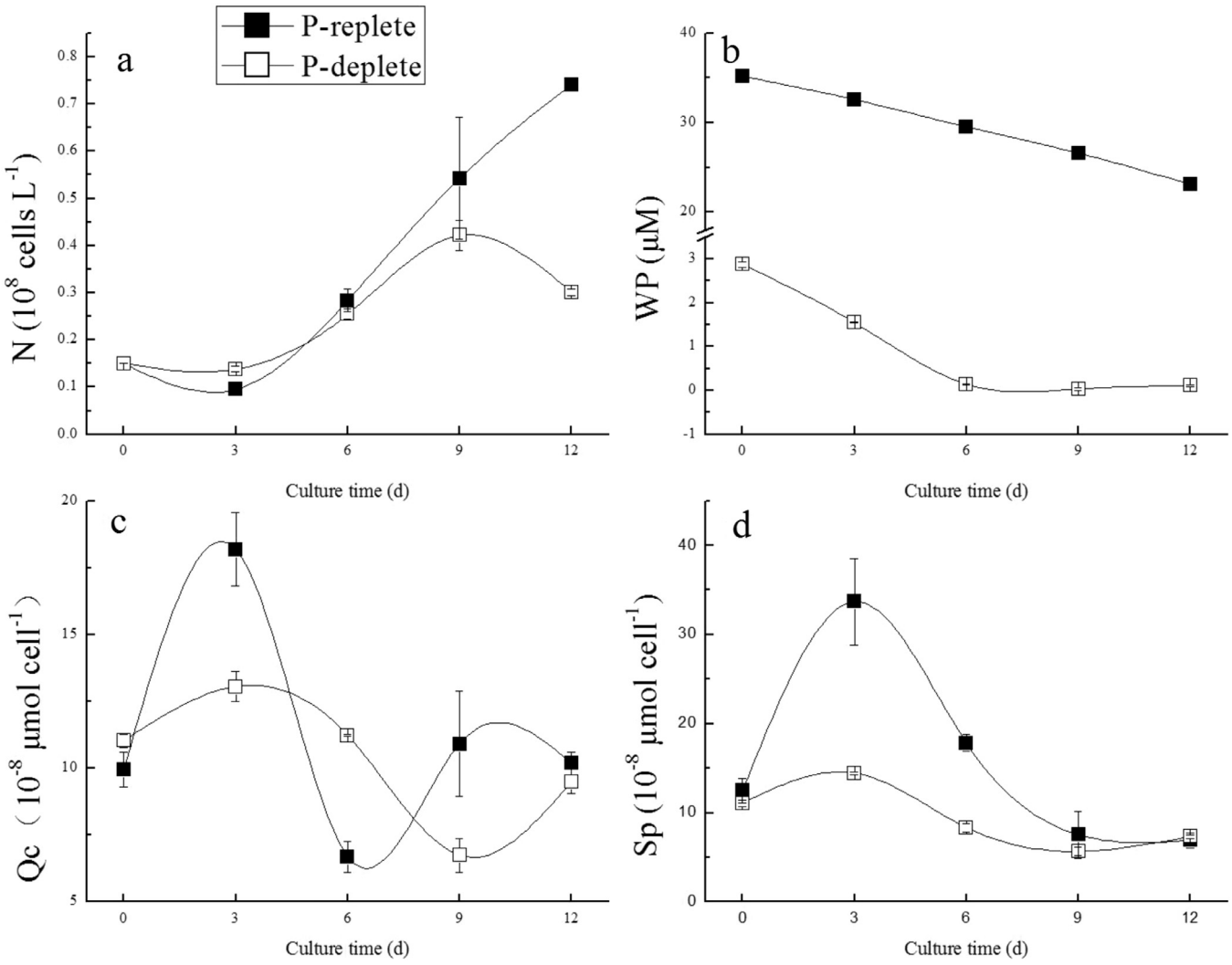

Fig. 3. Results of short-term P uptake experiments of *P. donghaiense* with replete P and deplete P at 24 °C. a: cell density (N); b: substrate P concentration (WP); c: cell quota of intracellular P (Q_c); d: cell quota of surface-adsorbed P (S_p).

tron transfer, Calvin Ring as well as the regulation of the activity of some enzymes (Wang et al., 2004; Shen and Li, 2016). In our study, the specific growth rate of *P. donghaiense* was obviously larger under P-replete condition than under P-deplete condition (Fig. 3a). Reason for this is that in the low-to-medium P concentration range, the highest cell density and relative growth rate are directly proportional to P concentrations and reach maxima at medium P concentrations (Long et al., 2005; Shen and Li, 2016). Under P-deplete condition, the end of the exponential growth phase of *P. donghaiense* reached earlier and came into the slow decline phase (Fig. 3a). Since the initial P limitation promotes the division of the algal cells, *P. donghaiense* under P-deplete condition first enters the exponential growth phase and quickly depletes the low concentration of P nutrient in the culture medium, reaches the highest cell density earlier than P-replete groups, and finally enters the slow decline phase.

Q_c in *P. donghaiense* under P-replete condition (Fig. 3c) increased more rapidly in the beginning than in group under P-deplete condition. The reason may be that algal cells under P-replete condition take less time to transport surface-adsorbed P into intracellular parts of cells than cells under P-deplete condition take. The periodic oscillations of Q_c in *P. donghaiense* under P-replete condition may be related to cell density of algae. Q_c in *P. donghaiense* under P-deplete condition did not show the periodic oscillations due to the fact that P concentration in substrate was too low to reflect the normal P uptake characteristics of *P. donghaiense*. The increase of S_p in *P. donghaiense* in the first 3 days showed the P transportation from substrate into surface-adsorbed parts of cells. S_p in *P. donghaiense* declined in the later days due to surface-adsorbed P from substrate was transported to intracellular part of cells gradually (Zhou, 2017).

Fitting data to one-stage model has indeed enhanced our understanding of P uptake characteristics of algae (Lang and Brown, 1981). However, until we know more about P uptake, it is unreasonable to use one-stage model in natural algal growth systems. Further experimental work must, to a greater extent, consider surface-adsorbed P and P concentration in substrate (Burmaster, 1979). Results suggest that the conventional one-stage P uptake model without thought of cell surface-adsorbed P also has a good fit to the experimental data, although it could not describe some details as well as the two-stage model. Both two models do not have notable differences between experimental data and fitted curves (Q_t and QP/ (AP + QP)) mathematically (Figs. 4 and 5). For example, both two-stage and one-stage models could fit the experimental data of *P. donghaiense* under P-deplete condition (Figs. 4b and 5b) very well. However, with careful comparison of the parameter values of the two models (Tables 5 and 6), the two-stage P uptake model is better able to explain the real P uptake

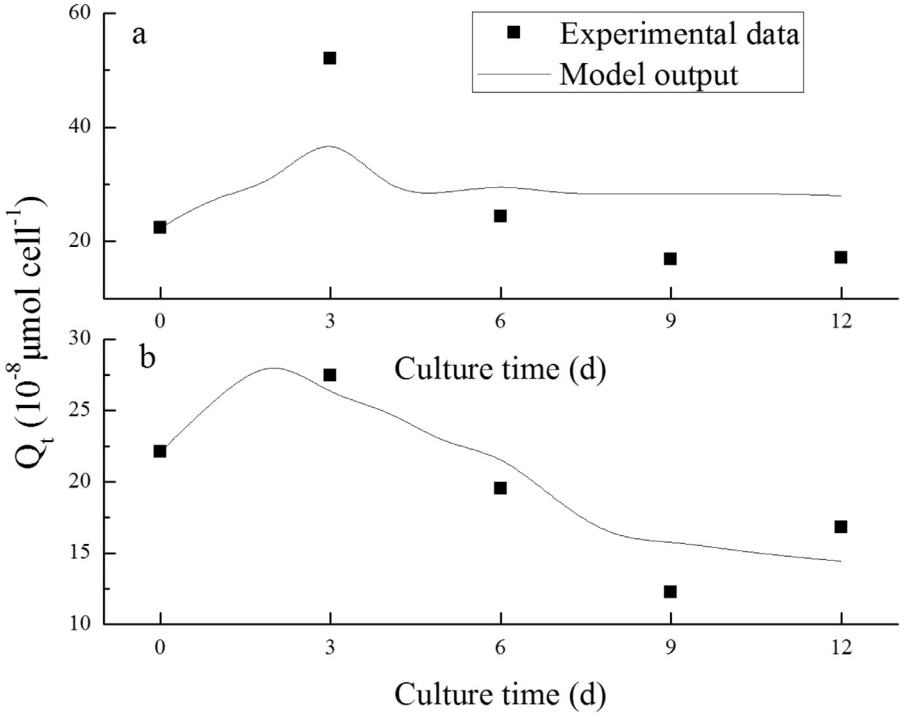

Fig. 4. Comparison of experimental data and fitted curve for Q_t of one-stage model in *P. donghaiense*. a: P-replete condition, b: P-deplete condition.

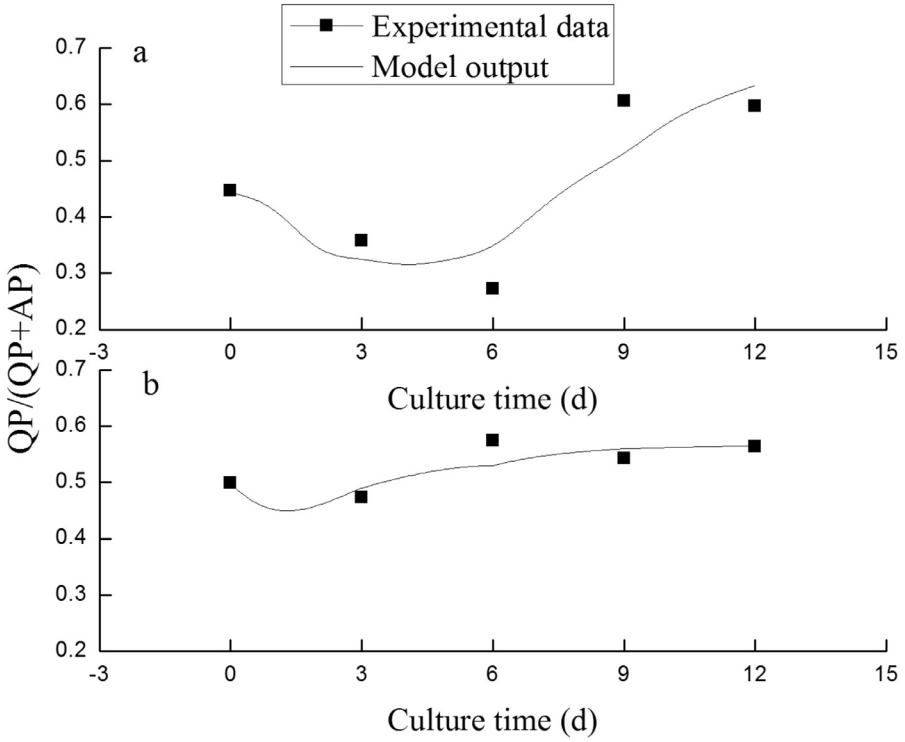

Fig. 5. Comparison of experimental data and fitted curve for QP/ (AP + QP) of two-stage model in *P. donghaiense*. a: P-replete condition, b: P-deplete condition.

process by algal cells under P-deplete and P-replete conditions, due to its parameters having more rational meaning and its curves fitting experimental data better than one-stage model in some cases. The parameter K_p represents the impact of P-starvation on P uptake rate, with a larger value denoting lower P concentration and larger P uptake rate per cell (Parslow et al., 1984; Litchman and Nguyen, 2008; Yao et al., 2011). In the two-stage uptake model, the estimated value of K_p in P-deplete group is larger than that in group under P-replete condition, which is in accordance with the definition of K_p (Table 5). However, in the one-stage uptake model, the estimated value of K_p appears not to represent P concentration as the group under P-replete condition has a much larger K_p value than the group under P-deplete condition (Table 6). Secondly, in some groups, the fitted curves of one-stage model does not match experimental data very well such as the group of *P. donghaiense* under P-replete condition at 24 °C (Fig. 4a), which shows to some

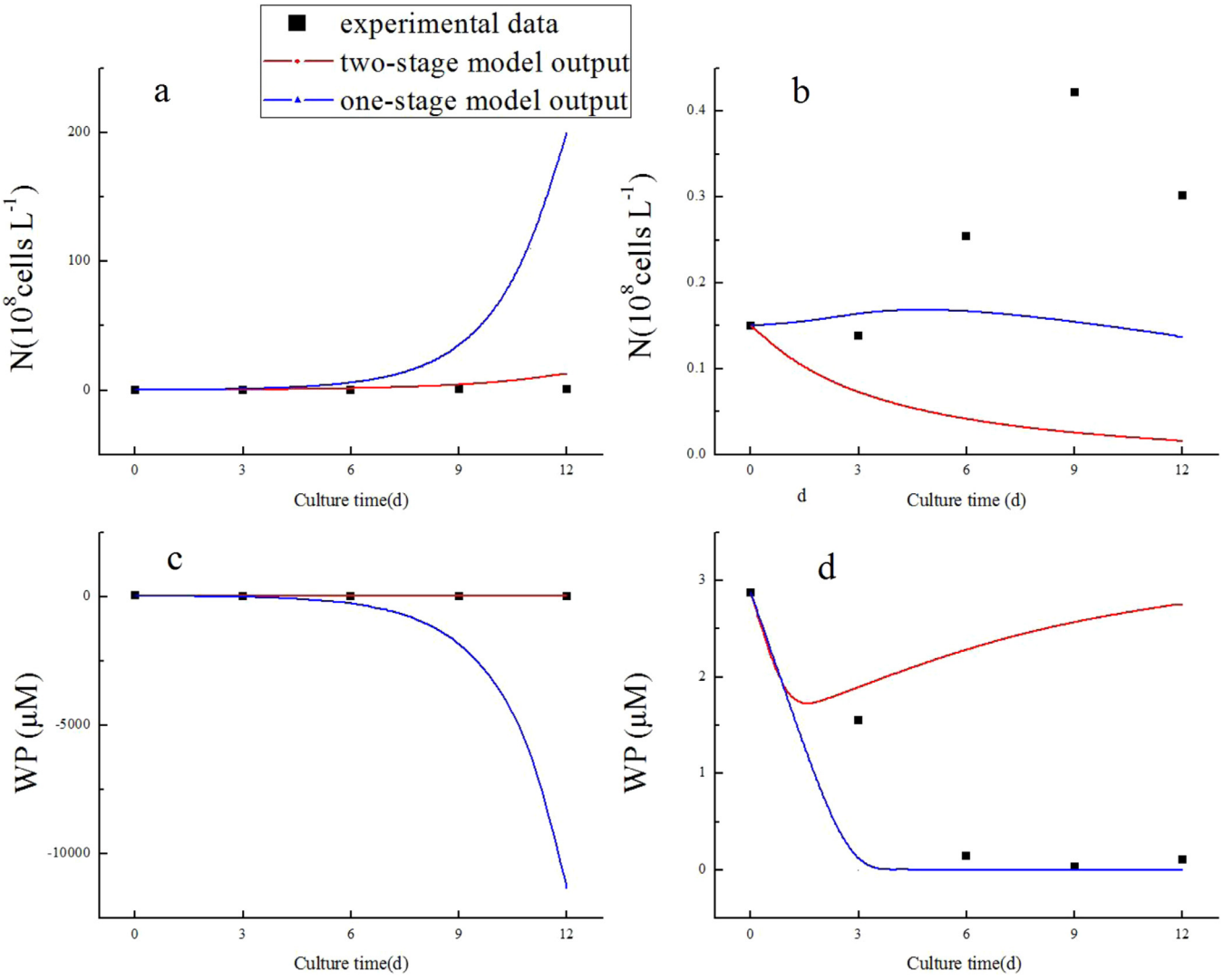

Fig. 6. Simulations of state variables N and WP of one-stage and two-stage models with corresponding experimental data. a: N under P-replete condition, b: N under P-deplete condition, c: WP under P-replete condition, d: WP under P-deplete condition.

extent, one-stage model is not capable to describe P uptake process.

Besides values of parameters and fitted curves in one-stage and two-stage models, Fig. 6 also shows two-stage model can better describe the P uptake process by algae. In Fig. 6, simulations of N and WP are performed under P-replete and P-deplete conditions by using one-stage and two-stage models. The initial values of the simulations are in accordance with experimental results on day 0, which are shown in Section 3.1 and the parameters used in simulations are listed in Tables 5–7. In Fig. 6a, the result that the simulation of N in one-stage model under P-replete condition breaks out over time is quite unreasonable. The simulation of WP in one-stage model under P-replete condition (Fig. 6c) becomes negative and decreases rapidly over time, which violates experimental data. In contrast, the simulations of WP and N in two-stage model under P-replete condition are close to experimental data (Fig. 6a and c). Under P-deplete condition, complicating environmental factors such as the presence of bacterial cells, fine debris and existing saturation of binding sites on algal surfaces may be important (Tien et al., 2005). Populations of *P. donghaiense* maintain high cell division rates under P-deplete condition by using DOP to augment DIP (Rivkin and Swift, 1985). These random factors are not considered in our two-stage model, so simulations of WP and N in two-stage model under P-deplete condition are not very close to experimental data (Fig. 6b and d). These results (Fig. 6) show the two-stage model could be used under P-replete condition well.

In addition, from the statistical point of view, two-stage model is more capable of describing characteristics of P uptake by algal cells than one-stage model. On one hand, AIC (Akaikes Information Criterion) is used to compare the distance from a candidate model to a “true” model (Burnham and Anderson, 2003). The model with higher AIC could better reflect dynamic process (Akaike, 1985). The value of AIC of one-stage model and two-stage model based on the experimental data of *P. donghaiense* under P-replete condition at 24°C are −74.35 and 44, while values of AIC under P-deplete condition are −70.578 and 54. Those results show that two-stage model is better suited to describe the P uptake process by algae. On the other hand, cross validation (Haefner, 1996) is used to compare models by splitting the data set into a training set and a test set. Using the training data, the model parameters are adapted (by means of a maximum log-likelihood procedure). Then, it is fair to compare maximal log-likelihood estimations directly: the model with higher maximal log-likelihood could better describe the kinetic process (de Vries et al., 2005). In the present case, every sin-

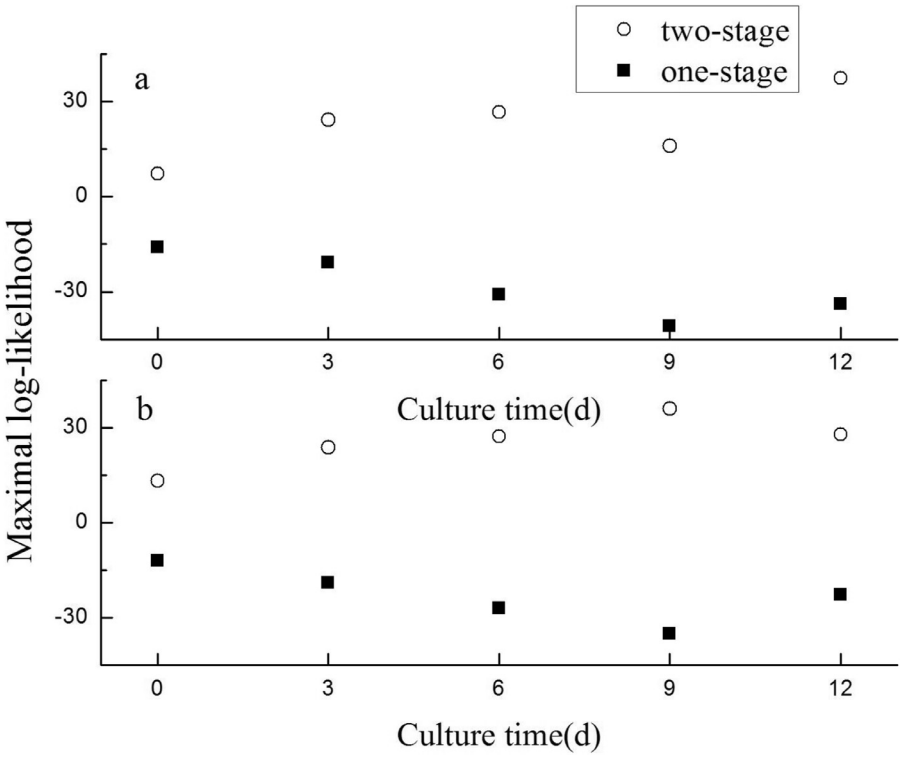

Fig. 7. Cross validation for one-stage model and two-stage model in *P. donghaiense*. Maximal log-likelihood estimations calculated by using experimental data of Q_t for one-stage model and QP/ (AP + QP) for two-stage model. a: P-replete condition, b: P-deplete condition.

gle experimental data of Q_t and QP/ (AP + QP) is used as the test set once, and the remaining data as the training set. Hence, we perform 5 maximal log-likelihood estimations for five data points. The results are shown in Fig. 7. We discover that maximal log-likelihood estimations for two-stage model are always larger than those for one-stage model, which implies that two-stage model is superior to one-stage model in the perspective of cross validation.

Experimental data Q_t and QP /(AP + QP) fitted by using one-stage and two-stage models respectively are of significance. Q_t represents the cell quota of intracellular P plus surface-adsorbed P, reflecting total P concentration per cell. QP/ (AP + QP) means proportion of intracellular P in total P of cells, emphasizing the importance of partitioning of the surface-adsorbed and intracellular P pools. In results of *P. donghaiense* (Figs. 4 and 5), whether the species under P-deplete condition or not, QP/ (AP + QP) decreased in the first few days, with corresponding slow increase of Q_t. This phenomenon could explain that S_p grew soon after *P. donghaiense* were put into media (Q_t increased, Fig. 4), while it took a few days for P on surface to transport into intracellular parts of cells (QP/ (AP + QP) decreased). As P was transported to intracellular part of cells, QP/ (AP + QP) started to increase (Fig. 5).

In conclusion, according to the experimental data of *P. donghaiense* under P-deplete and P-replete conditions at 24 °C, we obtain the parameter values of one-stage model and two-stage model and perform curve fitting. The results suggest that two-stage model could better describe P uptake process and the characteristics of P uptake by *P. donghaiense* are different under different P concentrations in substrate. To achieve the goal of predicting the outbreak process of algae in the ocean, based on the short-term and long-term observed data both in natural and laboratory environments, other factors such as temperature, pH, light intensity, water velocity and interactions between algae species (Zhang et al., 2018) need to be investigated to further clarify the entire dynamics of P uptake by algae.

Acknowledgments

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References

Akaike, H., 1985. A Celebration of Statistics. Springer, New York.
Aksnes, D.L., Egge, J.K., 1991. A theoretical model for nutrient uptake in phytoplankton. Mar. Ecol. Prog. Ser. 70, 65–72.
Anderson, D.M., 1997. Turning back the harmful red tide. Nature 388, 513–514.
Burmaster, D.E., 1979. The unsteady continuous culture of phosphate limited *Monochrysis lutheri* Droop: experimental and theoretical analysis. J. Exp. Mar. Biol. Ecol. 39, 167–186.
Burnham, K.P., Anderson, D.R., 2003. Model Selection and inference: a Practical Information Theoretic Approach. Springer, New York.
Droop, M.R., 1973. Some thoughts on nutrient limitation in algae. J. Phycol. 9, 264–272.
Droop, M.R., 1983. 25 years of algal growth kinetics: personal view. Botanica Marina 26, 99–112.
de Vries, G., Hillen, T., Lewis, M., Muller, J., Schonfisch, B., 2005. A Course in Mathematical biology: Quantitative Modeling With Mathematical and Computational Methods. University of Alberta, Edmonton.
Flynn, K.J., Fasham, M.J.R., Hipkin, C.R., 1997. Modelling the interactions between ammonium and nitrate uptake in marine phytoplankton. Philos. Trans. R. Soc. London Ser. B 352, 1625–1645.
Flynn, K.J., 2003. Modelling multi-nutrient interactions in phytoplankton; balancing simplicity and realism. Prog. Oceanogr. 56, 249–279.
Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Animals. Plenum Press, New York, pp. 26–60.

Goldman, J.C., Glibert, P.M., 1982. Comparative rapid ammonium uptake by four species of marine phytoplankton. Limnol. Oceanogr. 27, 814–827 LIMNOL OCEANOGR.
Gómez, F., 2005. A list of free-living dinoflagellate species in the world's oceans. Acta Bot. Croat. 64 (1), 129–212.
Granéli, E., Hansen, P.J., 2006. Alleopthy in harmful algae: a mechanism to compete for resources? In: Granéli, E., Turner, J.T. (Eds.) Ecology of Harmful Algae. Springer-Verlag, Berlin, pp. 189–201.
Harrison, P.J., Parslow, J.S., Conway, H.L., 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar. Ecol. Prog. Ser. 52, 301–312.
Haefner, J.W., 1996. Modeling Biological systems: Principles and Applications. Chapman and Hall, London.
John, E.H., Flynn, K.J., 2000. Modelling phosphate transport and assimilation in microalgae; how much complexity is warranted? Ecol. Model. 125, 145–157.
Karentz, D., Smayda, T., 1984. Temperature and seasonal occurrence patterns of 30 dominant phytoplankton species in Narragansett Bay over a 22-year period (1959–1980). Mar. Ecol. Prog. Ser. 18, 277–293.
Lang, D.S., Brown, E.J., 1981. Phosphorus-limited growth of a green alga and a blue-Green alga. Appl. Environ. Microb. 42, 1002–1009.
Lehman, J.T., Sandgren, C.D., 1982. Phosphorus dynamics of the prokaryotic nanoplankton in a Michigan lake. Limnol. Oceanogr. 27, 828–838.
Lu, D., Goebel, J., Qi, Y., Zou, J., Han, X., Gao, Y., Li, Y., 2005. Morphological and genetic study of *Prorocentrum donghaiense* Lu from the East China Sea, and comparison with some related *Prorocentrum* species. Harmful Algae 4, 493–505.
Long, H., Du, Q., 2005. Primary research on *K. mikimotoi* in Fujian coast. J. Fujian Fish. 12, 22–26 in Chinese with English abstract.
Litchman, E., Nguyen, B.L.V., 2008. Alkaline phosphatase activity as a function of internal phosphorus concentration in freshwater phytoplankton. J. Phycol. 44, 1379–1383.
Li, J., Glibert, P.M., Zhou, M., Lu, S., Lu, D., 2009. Relationships between nitrogen and P forms and ratios and the development of dinoflagellate blooms in the East China Sea. Mar. Ecol. Prog. Ser. 383, 11–26.
Lin, S., Litaker, R.W., Sunda, W.G., 2016. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. J. Phycol. 52, 10–36.
Morel, F.M.M., 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. 23, 137–150.
Parslow, J.S., Harrison, P.J., Thompson, P.A., 1984. Use of a self-cleaning in-line filter to continuously monitor phytoplankton nutrient uptake rates. Can. J. Fish. Aquat. Sci. 41, 540–544.
Riegman, R., Mur, L.R., 1984. Regulation of P uptake kinetics in *Oscillatoria agardhii*. Arch. Microbiol. 139, 28–32.
Rivkin, R.B., Swift, E., 1985. Phosphorus metabolism of oceanic dinoflagellates: phosphate uptake, chemical composition and growth of *Pyrocystis noctiluca*. Mar. Biol. 88, 189–198.
Roelke, D.L., Eldridge, P.M., Cifuentes, L.A., 1999. A model of phytoplankton competition for limiting and non-limiting nutrients: implications for development of estuarine and nearshore management schemes. Estuaries 22 (1), 92–104.
Sãnudo-Wilhelmy, S.A., Tovar-Sanchez, A., Fu, F., Capone, D.G., Carpenter, E.J., Hutchins, D.A., 2004. The impact of surface-adsorbed P on phytoplankton Redfield stoichiometry. Nature 432, 897–901.
Syrett, P.J., 1956. The assimilation of ammonia and nitrate by nitrogen-starved cells of *Chlorella vulgaris*. II. The assimilation of large quantities of nitrogen. Physiol. Plantarum 9, 19–27.
Saxton, M.A., Arnold, R.J., Bourbonniere, R.A., McKay, R.M., Wilhelm, S.W., 2012. Plasticity of total and intracellular phosphorus quotas in *Microcystis aeruginosa* cultures and Lake Erie algal assemblages.. Front. Microbio. 3, 3.
Shen, A., Li, D., 2016. Effects of different nutrients levels on the growth of *Procentrum donghaiense* and *Karenia mikimotoi*. Mar. Fish. 38 (4), 416–422 in Chinese with English abstract.
Shen, A., Ma, Z., Jiang, K., Li, D., 2016. Effects of temperature on growth, photophysiology, Rubisco Gene expression in *Prorocentrum donghaiense* and *Karenia mikimotoi*. Ocean Sci. J. 51 (4), 581–589.
Tovar-Sanchez, A., Sãnudo-Wilhelmy, S., Garcia-Vargas, M., Weaver, R.S., Popels, L.C., Hutchins, D.A., 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. Mar. Chem. 82, 91–99.
Tien, C., Sigee, D.C., White, K.N., 2005. Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics. J. Appl. Phycol. 17, 379–389.
Tang, D., Di, B., Wei, G., Ni, I., Oh, I., Wang, S., 2006. Spatial, seasonal and species variations of harmful algal blooms in the South Yellow Sea and East China Sea. Hydrobiologia 568, 245–253.
Wang, X., Deng, N., Zhu, C., 2004. Effect of nutrients (phosphate and nitrate) composition on the growth of HAB algae. Period. Ocean Univ. of China 34 (3), 453–460 in Chinese with English abstract.
Yao, B., Xi, B., Hu, C., Huo, S., Su, J., Liu, H., 2011. A model and experimental study of P uptake kinetics in algae: considering surface adsorption and P-stress. J. Environ. Sci. China 23 (2), 189–198.
Yu, X., Yuan, S., Zhang, T., 2018. The effects of toxin producing phytoplankton and environmental fluctuations on the planktonic blooms. Nonlinear Dyn 91 (3), 1653–1668.
Zhao, D., 2010. Occurrence Regularity of Marine Red Tide Disaster in Typical Areas in China. Marine Press, Beijing in Chinese.
Zhao, Y., Yuan, S., Zhang, T., 2016. The stationary distribution and ergodicity of a stochastic phytoplankton allelopathy model under regime switching. Commun. Nonlinear Sci. 37, 131–142.
Zhou, Q., 2017. Studies On Cellular Phosphorus Pool Characteristics of Dinoflagellates and Changes in Plankton Community During the Occurrence and Vanishment Process of Algal Blooms. Wenzhou University, Wenzhou in Chinese with English abstract.
Zhang, T., Liu, X., Meng, X., Zhang, T., 2018. Spatio-temporal dynamics near the steady state of a planktonic system. Comput. Math. Appl. 75, 4490–4504.