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典型赤潮藻对磷的生态生理响应

Ecophysiological Responses of Typical Harmful Algal Bloom Species to Phosphorus

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缩写语对照表

5’-PN：5’-nucleotidase，5－核苷酸酶

AP：Alkaline phosphatase，碱性磷酸酶

APA：Alkaline phosphatase activity，碱性磷酸酶活性

ATP：Adenosine triphosphate，三磷酸腺苷

DIN：Dissolved inorganic nitrogen，溶解无机氮

DNA：Deoxyribonucleic acid，脱氧核糖核酸

DO：Dissolved oxygen，溶解氧

DOC：Dissolved organic carbon，溶解有机碳

DON：Dissolved organic nitrogen，溶解有机氮

DOP：Dissolved organic phosphorus，溶解有机磷

ELF：Enzyme labeled fluorescence，酶标记荧光

G-6-P：Glucose-6-phosphate，葡萄糖－6－磷酸

HABs：Harmful Algal Blooms，有害藻华（赤潮）

LEC：Lecithin，卵磷脂

MFP：3-0-methylfluorescein phosphate，3-0-甲基荧光素磷酸盐

P：Phosphorus，磷

P_i：Orthophosphate，正磷酸盐

PP：Particulate phosphorus，颗粒磷

RNA：Ribonucleic acid，核糖核酸

SNP：Soluble nonreactive phosphorus，溶解态非活性磷

SRP：Soluble reactive phosphorus，溶解态活性磷

TDN：Total dissolved nitrogen，总溶解态氮

TDP：Total dissolved phosphorus，总溶解态磷

摘　　要

采用现场调查、现场船基围隔和室内培养实验等手段，研究中国近海若干海区浮游植物的磷营养限制与胁迫状况，选取几种典型赤潮藻东海原甲藻（*Prorocentrum donghaiense*）、链状亚历山大藻（*Alexandrium catenella*）、中肋骨条藻（*Skeletonema costatum*），研究该赤潮藻对营养盐（主要为磷）的生态生理响应，探讨赤潮藻的磷竞争策略，分析赤潮藻在磷营养代谢上的竞争优势及其机制。主要实验结果如下：

1．现场碱性磷酸酶（Alkaline phosphatase, AP）结果表明，东海长江口邻近海域赤潮高发区、台湾海峡的南部海区、厦门港等研究海域都存在不同程度的磷胁迫。其中，又以长江口邻近海域赤潮高发区的磷胁迫状态最为严重。结合现场营养盐、碱性磷酸酶活性（Alkaline phosphatase activity, APA）及营养盐加富围隔实验数据，表明, 春季长江口赤潮高发区内浮游植物的生长受到磷营养盐的限制。台湾海峡南部近岸上升流区，水华发生导致浮游植物的磷胁迫加剧。

2002 年、2003 年和 2005 年春季（4 ~ 5 月），长江口邻近海域赤潮高发区内，浮游生物群落存在磷胁迫状况（2005 年春季长江口外近岸水域除外），这一胁迫程度在 2003 年春季东海原甲藻赤潮爆发期间有所加剧。APA 粒径结果表明，浮游植物是 AP 主要的提供者。2005 年春季，高比例的酶标记荧光（ELF%）表明优势种甲藻受到显著磷胁迫，是该海区 AP 的主要贡献者，硅藻和金藻几乎不存在磷胁迫。

2004 年夏季（7 ~ 8 月），台湾海峡南部海区，浮游植物群落受到磷胁迫。在近岸上升流区内，水华发生导致浮游植物的磷胁迫程度加深。高比例 ELF% 表明甲藻受到严峻的磷胁迫，此外优势种硅藻，如冰河拟星杆藻（*Asterionellopsis glacialis*）、菱形海线藻（*Thalassionema nitzschioides*）和尖刺拟菱形藻（*Pseudo-nitzschia pungens*）的磷胁迫程度也较为严峻。2005 年冬季（3 月），海区浮游植物的磷胁迫程度有所缓解。

2．海区 AP 调控机制非常复杂，受多因子（包括温度、盐度、pH、溶解氧、营养盐的浓度与比例、浮游植物群落结构及其丰度）共同作用，且不同因子的作用效力不是一定的，随海区实际情况有所改变。多数情况下，营养盐是调控

AP 的重要因子，海区 APA 随营养盐浓度的降低而显著升高。

3．当环境中磷缺乏时，因具备较低的半饱和常数（K_s），中肋骨条藻是最有利的磷酸盐竞争者，其次为东海原甲藻；当环境中以脉冲方式输入大量的磷酸盐时，因具备较高的最大吸收速率（ρ_{max}），链状亚历山大藻为磷酸盐最有利的竞争者，其次为东海原甲藻。

4．单位细胞链状亚历山大藻的磷需求高于东海原甲藻和中肋骨条藻。三种赤潮藻均具有磷储库，但是在大规模生长繁殖时，即使环境中磷酸盐尚未耗尽，藻细胞内的磷储库也迅速下降。三种赤潮藻的磷储库都不足以在环境磷胁迫的情况下，长时间维持细胞的增长。

5．在补充相同磷源的情况下，中肋骨条藻的细胞生长数目最高，其次为东海原甲藻和链状亚历山大藻。在营养盐较为丰富的海区，起始阶段，中肋骨条藻生长速率最快，相较于东海原甲藻和链状亚历山大藻更易大规模增殖形成赤潮。东海原甲藻和链状亚历山大藻能长时间在贫营养环境中保持细胞的生理活性，并且在利用溶解态非活性磷（Soluble nonreactive phosphorus, SNP）上较有优势。因此，在中肋骨条藻的衰亡期，海区 SNP 含量上升或是海区补充新的磷源的情况下，东海原甲藻和链状亚历山大藻可能竞争过中肋骨条藻形成赤潮；与链状亚历山大藻相比，东海原甲藻因个体小，对磷的需求小，生长数目能远远超过链状亚历山大藻。

6．混合培养的情况下，不论是改变无机态正磷酸盐（P_i）的浓度，或是以不同形态的有机磷为磷源，中肋骨条藻的细胞生长都能在短时间内超过东海原甲藻，成为绝对的优势种。因此，当海区同时存在东海原甲藻和中肋骨条藻，且细胞都保持较高活性的情况下，海区磷营养盐的改变不是导致原甲藻取代骨条藻成为优势种的主要原因。当混合培养体系中东海原甲藻与链状亚历山大藻细胞数目相当时，链状亚历山大藻可能通过相克作用抑制东海原甲藻的生长，导致原甲藻死亡。不同形态的有机磷源对东海原甲藻与链状亚历山大藻的竞争生长影响较大，大分子的溶解有机磷源有利于东海原甲藻的生长。

7．东海原甲藻、链状亚历山大藻、中肋骨条藻的 AP 均为诱导酶，在藻类遭受磷胁迫时产生，三种赤潮藻均不存在结构酶。三种赤潮藻能依赖溶解有机磷为磷源维持生长。AP 水解 SNP 的能力强，只需要少量酶作用就能迅速水解

水体中大量的 SNP。赤潮藻在磷胁迫的情况下，会过量诱导 AP 产生，消耗能量。

8．东海原甲藻 AP 位点主要分布在细胞表面，少量位于细胞内部。当遭受严峻磷胁迫时，AP 位点遍布整个细胞表面，最大 APA = 12.84 fmol $cell^{-1}$ h^{-1}。AP 产生后，会被逐步释放到水体中；链状亚历山大藻 AP 位点主要分布于细胞内部，细胞表面也有分布。当遭受严峻磷胁迫时，AP 位点遍布整个细胞，最大 APA = 66.77 fmol $cell^{-1}$ h^{-1}，释放 AP 的速度较为缓慢；中肋骨条藻 AP 位点分布于细胞表面。当遭受严峻磷胁迫时，细胞表面出现 1 至 2 个 AP 位点，最大 APA = 1.62 fmol $cell^{-1}$ h^{-1}，AP 产生后，会被迅速释放到水体中。

9．东海原甲藻具有高亲和力的 AP 水解系统，而中肋骨条藻与链状亚历山大藻没有。当环境中磷酸盐耗尽且存在较低含量的 SNP 时，东海原甲藻 AP 因具有较低的半饱和常数（K_s），对底物的亲和力较高，较有竞争优势。当环境中存在大量的 SNP 时，链状亚历山大藻 AP 具有较高的水解速率（V_{max}），较有竞争优势。

关键词：典型赤潮藻，磷，溶解态非活性磷，生态生理策略，碱性磷酸酶

Abstract

The study on ecophysiological responses of typical HAB species to nutrients (in particular phosphorus) were carried out using field survey, ship-based mesocosm experiments and lab-based incubation experiments in the high frequency HAB areas in the Yangtze River estuary and its adjacent East China Sea, southern Taiwan Strait and Xiamen Harbour. The phosphorus status of natural phytoplankton and the effects of nutrients on phytoplankton community structure were evaluated. The strategies of typical HAB species to the variations of environmental phosphorus were emphasized on, and whether the typical HAB species advantaged in competing for phosphorus and its mechanisms were discussed. The main results were as follows:

1. Alkaline phosphatase activity (APA) showed that phytoplankton in the study areas suffered phosphorus (P) stresses with different degrees, with significance during bloom occurrence in sea areas in the Yangtze River estuary. All results from nutrients, APA and mesocosm enrichment experiments on board indicated that phytoplankton was P limited in the high frequency HAB areas in the Yangtze River estuary during spring. In the bloom period at the coastal upwelling zone in southern Taiwan Strait during summer, phytoplankton's P-stress status strengthened.

During the spring (April ~ May) of 2002, 2003 and 2005, phytoplankton communities were under P stress in the Yangtze River estuary and its adjacent East China Sea (except the area just outside the Yangtze River estuary during the spring of 2005). P stress degrees strengthened during the bloom period in 2003. Size - fractionated APA indicated that phytoplankton was the main contributor of total APA. During the spring of 2005, high percentages of ELF labeling of the dominant dinoflagellates suggested that they suffered severe P stress and were the main contributors of AP while very low percentages of ELF labeling were found among Chrysophyta and diatom, indicating that they were not P stressed or slightly P stressed.

During the summer (July ~ August) of 2004, phytoplankton community suffered P stress in southern Taiwan Strait, in particular during the bloom period in the coastal upwelling zone. High percentages of ELF labeling of dinoflagellates showed that they were under severe P-stress status. Besides, the dominant species diatom, such as

Asterionellopsis glacialis, *Thalassionema nitzschioides* and *Pseudo-nitzschia pungens* also suffered P stress somewhat. The phytoplankton P-stress status softened during the winter of 2005 (March).

2. The mechanisms on APA were integrated. APA was influenced by many factors, such as temperature, salinity, pH, dissolved oxygen, nutrient concentrations and their ratios, phytoplankton community structure and abundances. The effectiveness of factors shifted with the variation of natural condition. In most condition, nutrients were the significant factors for APA, APA increased with the decrease of nutrient concentrations.

3. When phosphate was depleted, *Skeletonema costatum* was advantaged in gaining phosphate due to the low constant of uptake kinetics (K_s), and then came to *Prorocentrum donghaiense*. When pulses of abundant phosphate were imported into the system, *Alexandrium catenella*, owing to the high uptake rate (V_{max}), was advantaged in acquiring phosphate, and then came to *P. donghaiense*.

4. The specific cell P quota of *A. catenella* was higher than that of *P. donghaiense* and *Sk. costatum*. All three test HAB species had the ability to storage P (P pool) inside the cell. However, when phytoplankton grew exponentially, even the phosphate was not exhausted, the intracellular P pool decreased rapidly. The intracellular P pool of the test HAB species could not sustain their growth for a longer time under P-stress status.

5. When supplying the same amount of phosphate, *Sk. costatum* could get the highest cell numbers, and then came to *P. donghaiense* and *A. catenella* in order. Under the eutrophication condition, the growth rate of *Sk. costatum* was highest, advantaged in bloom comparing with *P. donghaiense* and *A. catenella*. With the depletion of phosphate, cell number of *Sk. costatum* decreased abundantly. However, *P. donghaiense* and *A. catenella* could keep the cellular physiological activities and abundances at a certain extent and were advantaged in utilizing soluble nonreactive phosphorus (SNP) comparing with *Sk. costatum*. When stimulated with pulse nutrients or SNP increased in the system, the growth rates of *P. donghaiense* and *A. catenella* surpassed that of *Sk. costatum* and might bloom in this period. *P.*

donghaiense was advantaged in smaller cell size and P requirement comparing with *A. catenella* and the cell number of *P. donghaiense* could out-compete that of *A. catenella*.

6. Under the co-exist incubation experiment of *P. donghaiense* and *Sk. costatum*, the variation of P (whatever the phosphate concentration or the forms of P compound) didn't changed the competing result that the cell growth of *Sk. costatum* always exceeded that of *P. donghaiense* in a short time and *Sk. costatum* became the absolutely dominant species. Therefore, when *P. donghaiense* and *Sk. costatum* co-existed in the natural environment and both cells kept relative high physiological activities, the variation of P couldn't be the key factor that makes *P. donghaiense* out-competing *Sk. costatum* to bloom. As for the co-exist culture of the same amount of *P. donghaiense* and *A. catenella*, *A. catenella* might restrain the growth of *P. donghaiense* through allopathy and *P. donghaiense* died after some days. Variation of forms of P compounds might impress a lot on the growth competition of these two species. The existence of larger molecular SNP might favor the growth of *P. donghaiense*.

7. The alkaline phoshatase (AP) of *P. donghaiense*, *A. catenella* and *Sk. costatum* were inducible, inducing by the algal cells under P stress. No constitutive enzyme was found among the test HAB species. All three test HAB species could grow using SNP as the sole P source. AP was efficient in hydrolyzing SNP, and just some nmol l^{-1} h^{-1} APA could hydrolyze abundant SNP in a short time. Under P-stress status, these species would produce excess AP and exhausted abundant energy for growth.

8. AP sites of *P. donghaiense* were mainly on the cell surface and some could be found inside the algal cells. When suffered severe P stress, AP of *P. donghaiense* covered all around the cell surface and the maximum specific APA could get as high as 12.84 fmol $cell^{-1}$ h^{-1}. *P. donghaiense* would released AP into the water (by free) gradually; AP sites of *A. catenella* was mainly inside the cell and some could be found on the cell surface. When suffered severe P stress, AP of *A. catenella* covered all around the cell and the maximum specific APA could get as high as 66.77 fmol