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Application of qPCR methods in detection of PST-producing *Alexandrium* species in the Yellow Sea and East China Sea

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Background

- Some dinoflagellate species within the genus *Alexandrium* produce potent phycotoxins including paralytic shellfish toxins (PSTs). Blooms of these toxic *Alexandrium* species pose substantial hazards to human health.

- Among the members within the *Alexandrium* genus, *A. tamarense* 'species complex' (i.e. *A. tamarense*, *A. catenella*, and *A. fundyense*) are the most extensively studied.

- Recently, taxonomy of *A. tamarense* 'species complex' were revised to identify it as five distinct species based on their conserved differences between their rDNA sequences (John et al. 2014). The species cannot be simply discriminated by their morphology, and qPCR methods based on their unique rDNA sequences showed great advantages in ecological studies of these species.

- A qPCR assay targeting *sxtA*4, a domain in the *sxt* gene cluster that encodes a unique enzyme involved in STX biosynthesis, has been developed for detection of toxic algae associated with PSTs (Murray et al. 2011).

- In past investigations along the Chinese coast, species of *A. tamarense* 'species complex' have been distinguished as either ''*A. tamarense*'' or ''*A. catenella*'' based on their morphological features, though genetic studies have shown that both *A. fundyense* (Group I) and *A. pacificum* (Group IV) commonly occur.

- Shellfish cultivated in the Yellow Sea (YS) and East China Sea (ECS) are often contaminated by PSTs, probably associated with toxic *Alexandrium* spp.

- We applied the qPCR assays in together with a post-column HPLC method for detection of PSTs, to test their feasibility in monitoring toxic algae in the YS and ECS.

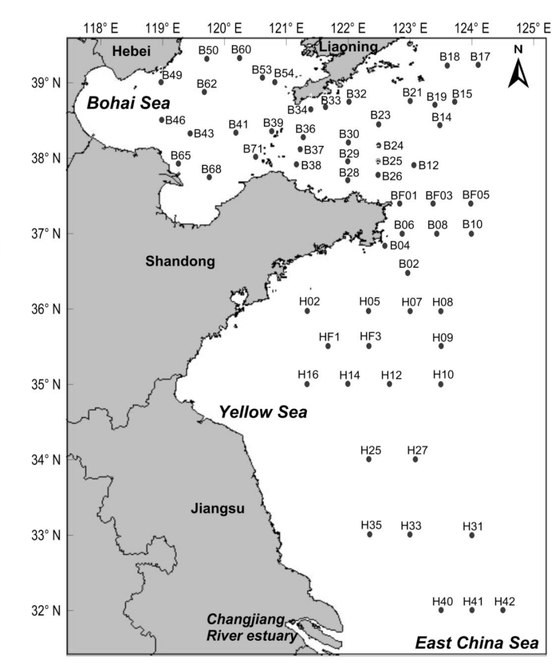


Illustration of the sampling sites in the Bohai Sea (BS), Yellow Sea (YS) and East China Sea (ECS)

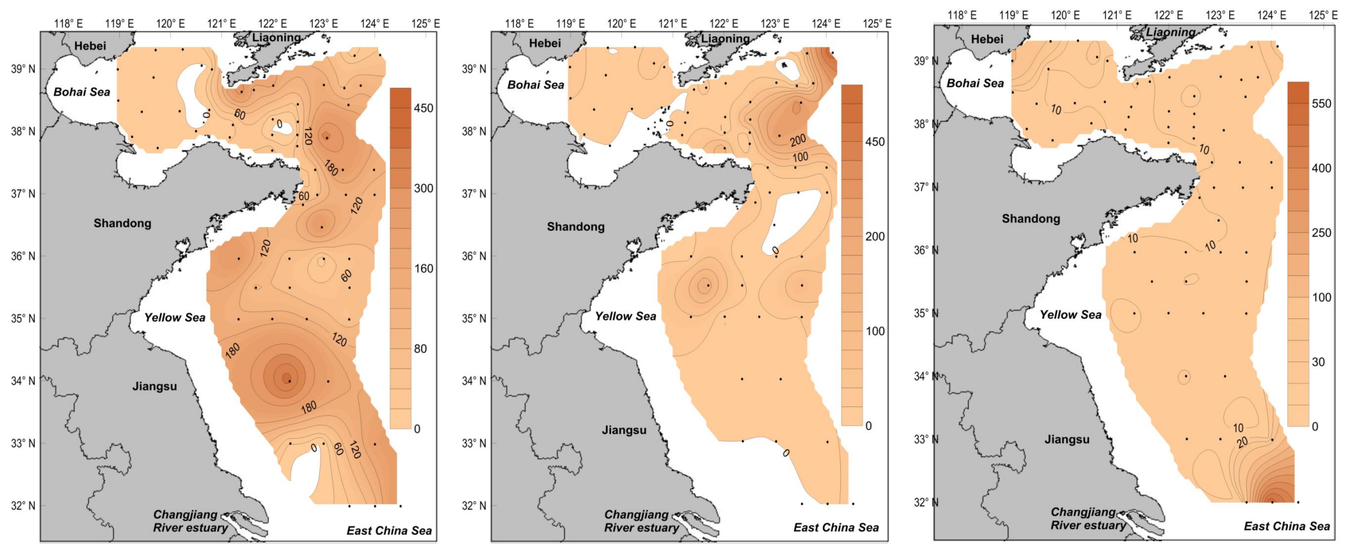
Materials and Methods

- Net-concentrated (20mm) samples collected in a cruise organized by National Natural Science Foundation of China during May 12–20 in 2012 were analyzed.
- Total *Alexandrium* cells were counted under a light microscope.
- Samples were analyzed a qPCR assay targeting *sxtA*4 (a domain in the *sxt* gene cluster for STX biosynthesis) and two TaqMan based qPCR assays for *A. fundyense* and A. *pacificum*.
- PSTs in net-concentrated were determined with high-performance liquid chromatography coupled with a fluorescence detector using a post-column method.

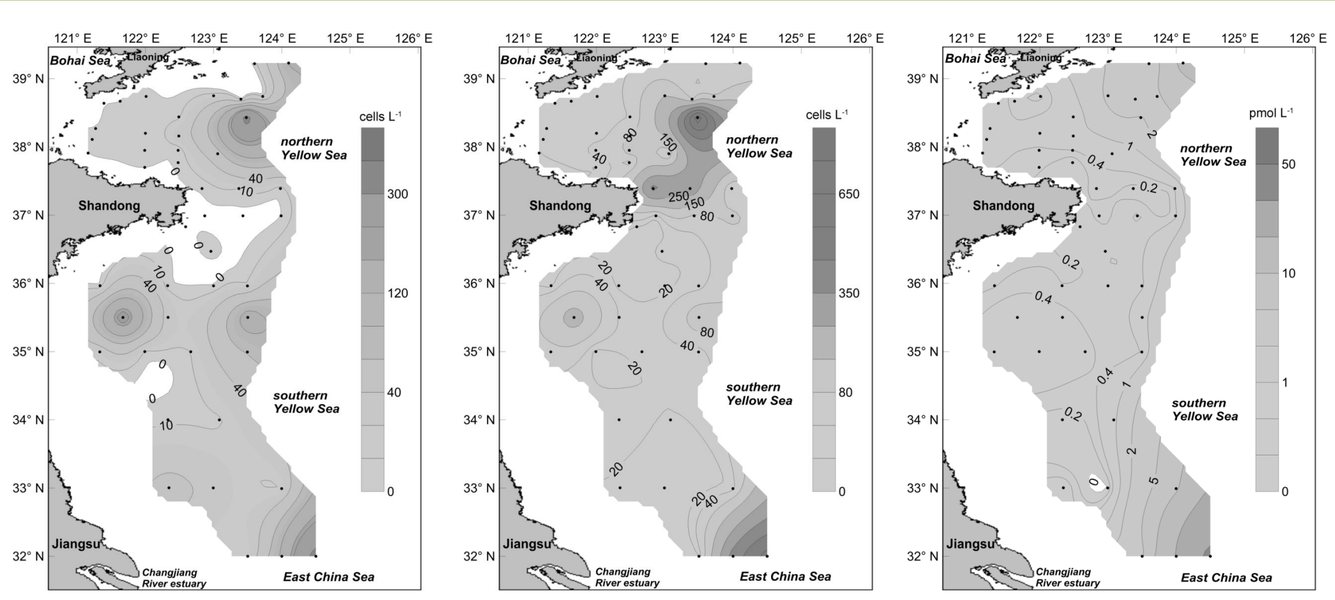
Results

- Distribution of two toxic *Alexandrium* species

- *Alexandrium* spp. were present throughout both the YS and BS, but were concentrated within YS in Utermöl's cell counts of samples taken during the May survey.
- *A. fundyense* and *A. pacificum* showed distinct distribution patterns in the study area.
- *A. fundyense* were most abundant in the central part of northern YS.
- The distribution of *A. pacificum* was confined to the joint area between the YS and ECS.
- The different distribution of *A. fundyense* and *A. pacificum* within the region is likely reflect significant differences in their ecology, especially the distribution of their resting cysts.



Distribution patterns of total *Alexandrium* cells (left), *A. fundyense* (middle) and A. *pacificum* (right)



Distribution patterns of *sxtA*4 gene (left), toxic algae (*A. fundyense+A. pacificum,* middle) and PSTs (right)

- Feasibility of the qPCR assay in monitoring toxic algae associated with PSTs

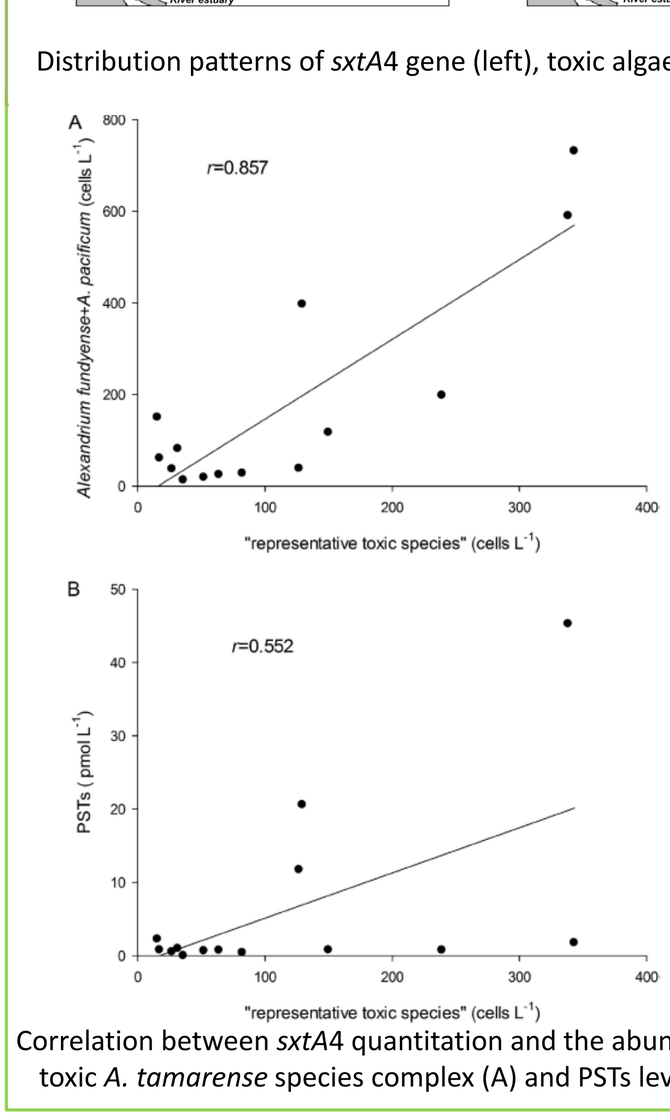
- The distribution of the *sxtA*4 gene in the YS was consistent with the toxic algae and PSTs.

- The quantitation results of *sxtA*4 correlated well with the abundance of the two toxic species.

- It was suggested that the two toxic species (*A. fundyense* and *A. pacificum*) were major PST producers during the sampling season in YS and ECS.

- The sxtA-based qPCR is a promising method to detect toxic algae associated with PSTs in the YS.

- The *sxtA*-based qPCR is not accurate enough to reflect the toxicity of PST producing toxic algae.



Correlation between *sxtA*4 quantitation and the abundance of toxic *A. tamarense* species complex (A) and PSTs levels (B).

Conclusion

-Different qPCR assays have been applied to study the distribution of toxic *A. fundyense* and *A. pacificum* in YS and ECS. It was found that *A. fundyense* distributed mainly in the sea area north to the latitude 34°N and was the most dominant specie in northern YS. *A. pacificum* was mainly confined to the area near the Changjiang River estuary.

- An *sxtA*-based qPCR assay was applied to study toxic algae associated with PSTs in the YS and proved to be a promising method. The qPCR assay showed high specificity for detecting PST-producing microalgae, and the quantitation results of *sxtA4* represented well the abundance and distribution of the toxic *A. tamarense* species complex (*A. fundyense and A. pacificum)* in the YS during the sampling season.

Acknowledgements

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For more information:

- Gao Y, Yu RC, Murray SA, Chen JH, Kang ZJ, Zhang QC, Kong FZ, Zhou MJ. 2015. High Specificity of a Quantitative PCR Assay Targeting a Saxitoxin Gene for Monitoring Toxic Algae Associated with Paralytic Shellfish Toxins in the Yellow Sea. ***Applied and Environmental Microbiology***, 81(20): 6973-6981.
- Gao Y, Yu RC, Zhang QC, Kang ZJ, Chen JH, Wang YF, Kong FZ, Yan T, Zhou MJ. , 2015. Distribution of *Alexandrium fundyense* and *A. pacificum* (Dinophyceae) in the Yellow Sea and Bohai Sea. ***Marine Pollution Bulletin***, 96:210-219.