Monitoring Toxic HAB in Chinese waters (Jiaozhou Bay & Zhoushan islands) during the recent three years

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Introduction

Toxic Harmful Algal Blooms (HABs) have been spreading and increasing along the Chinese coast during the past few years, causing damage to mariculture as well as human health.

- PSP (Paralytic Shellfish poisoning) and DSP (Diarrhetic Shellfish Poisoning) are most often occurred
- Ciguatera Fish Poisoning has also been found and increased in the southern China

HAB toxin investigations during the 90's

- PSP occurs most frequently in south of China, and occasionally occurs in the north and middle of China
- DSP has been found widely distributed along the Chinese coast, with a higher amount in the north than in the south.

This paper focuses on two recent toxic HAB monitoring :

- Jiaozhou Bay monitoring program during 2001-2003
- Zhoushan islands spring HAB investigation during 2002-2004


RUSSIA
KAZAKHSTAN
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JILIN
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INNER MONGOLIA
Beijing
GANSU
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SHANDONG
Yellow River
CHINA
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TIBET
ANHUI
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NEPAL
SICHUAN
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BHUTAN
Yangtze River
East
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BANGLADESH
YUNNAN
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TAIWAN
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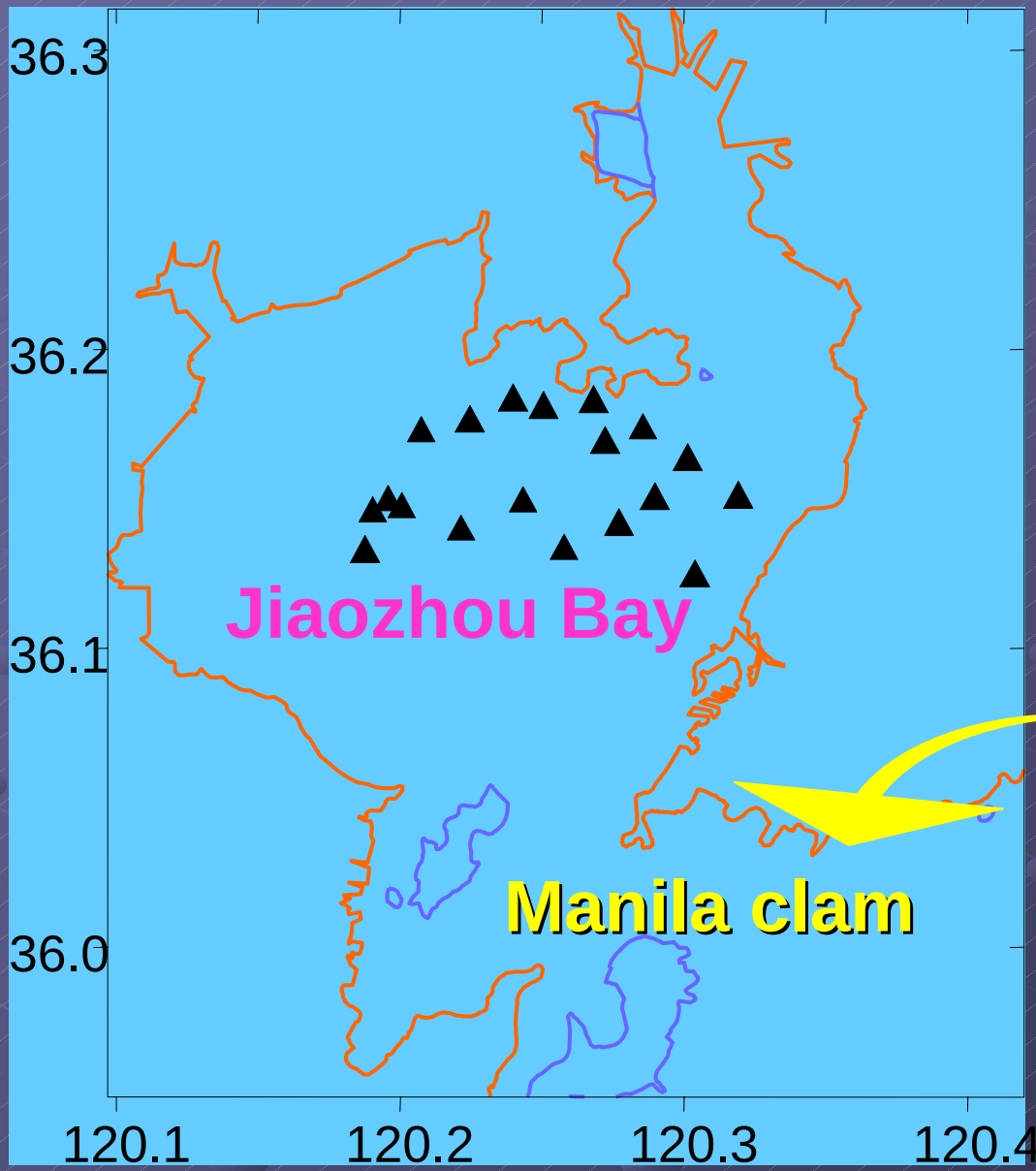
SCMP Graphic
Jiaozhou Bay
Zhoushan islands

Jiaozhou Bay monitoring program during 2001-2003



- Monitoring season:

  Once a month from May to September each year, this is increased to weekly when HAB toxin is found in shellfish

- Monitoring program:

  Phytoplankton, especially toxic algae sampling, identification and quantification;

  shellfish sampling, toxin determination (PSP and DSP) by mouse bioassay, and further HPLC analysis if the mouse assay shows positive results.

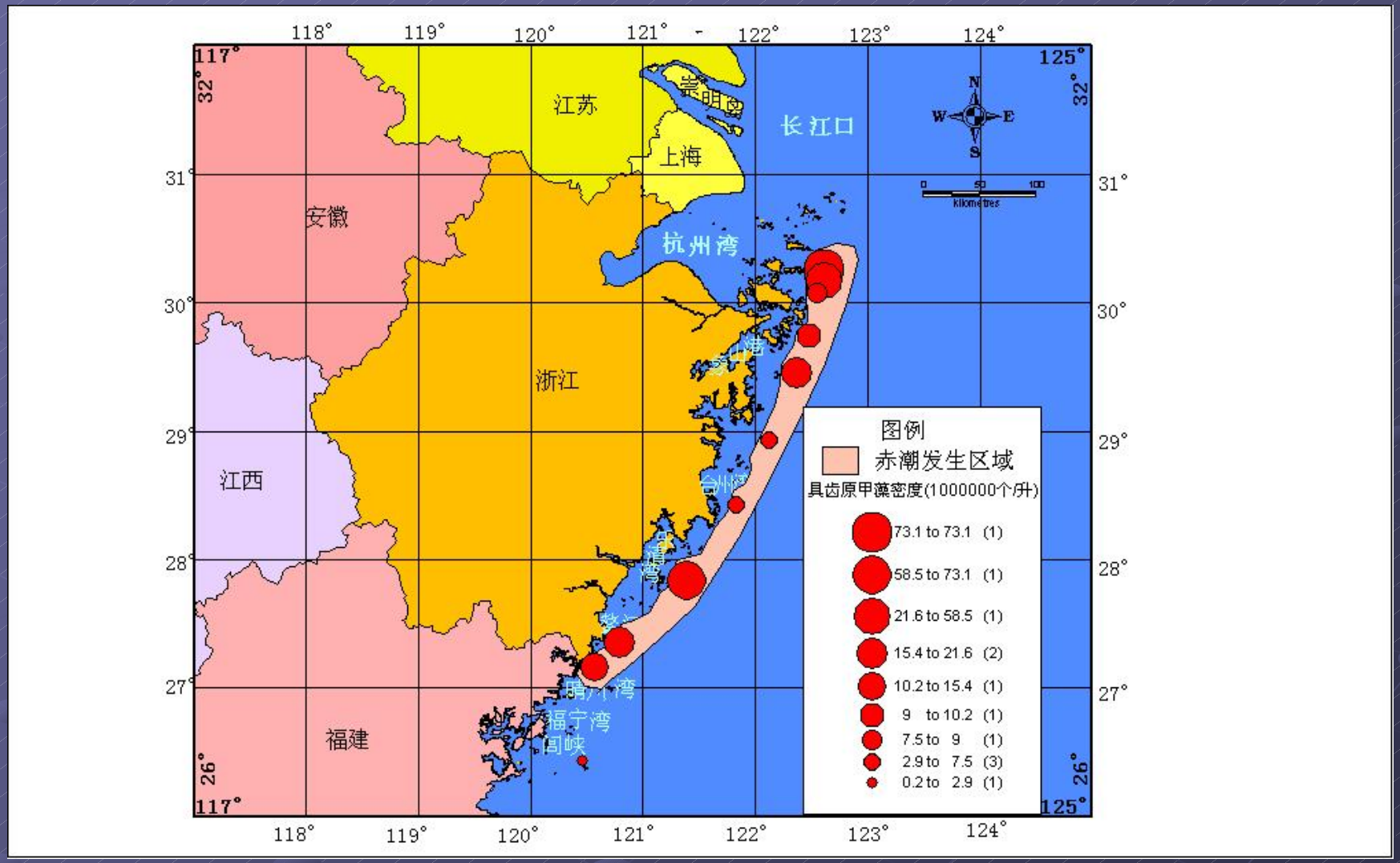
Zhoushan area spring HAB investigation during 2002-2004

Background

- East China Sea is the most serious HAB area in China in the past few years. Spring red tide with a large area occurred continuously for 5 years. During May of 2002 and May of 2004, large and extremely large scale (over 10,000km^2) red tide occurred in Zhoushan area of East China Sea with the causative algae of *Prorocentrum* and *Alexandrium*. The happening of red tide caused harmful impact to fishery and people's life, especially hearing the report said that *Alexandrium* could produce deadly PSP toxins which could accumulate in shellfish.

Date	Location	Area(m^2)	HAB causative algea
MAY3−24, 2000	Zhongjieshan islands, Zhoushan	7000	*Prorocentrum dentatum /Alexandrium*
May 10-17, 2001	Huaniaoshan area, outside of the Changjiang River estuary	1000	*Prorocentrum dentatum*
May, 10-13,2001	Zhongjieshan islands, Zhoushan	3000	*Prorocentrum dentatum /Alexandrium*
May 3-11, 2002	Zhongjieshan islands, Zhoushan	800	*Prorocentrum dentatum /Alexandrium*
May 17-19, 2002	Shengsi islands	900	*Prorocentrum dentatum / Skeletonema costatum /Mesodinium rublum*
May 4-11, 2003	Zhongjieshan islands, Zhoushan	3000	*Prorocentrum dentatum*
June 25-30, 2003	Sea area outside of the Changjiang River estuary	1000	*Prorocentrum dentatum / Skeletonema costatum*
May 3-20, 2004	Middle and south sea area of Zhejiang province	**10000**	*Prorocentrum dentatum /Alexandrium*

Spring HAB events in the East China Sea during 2000~2004



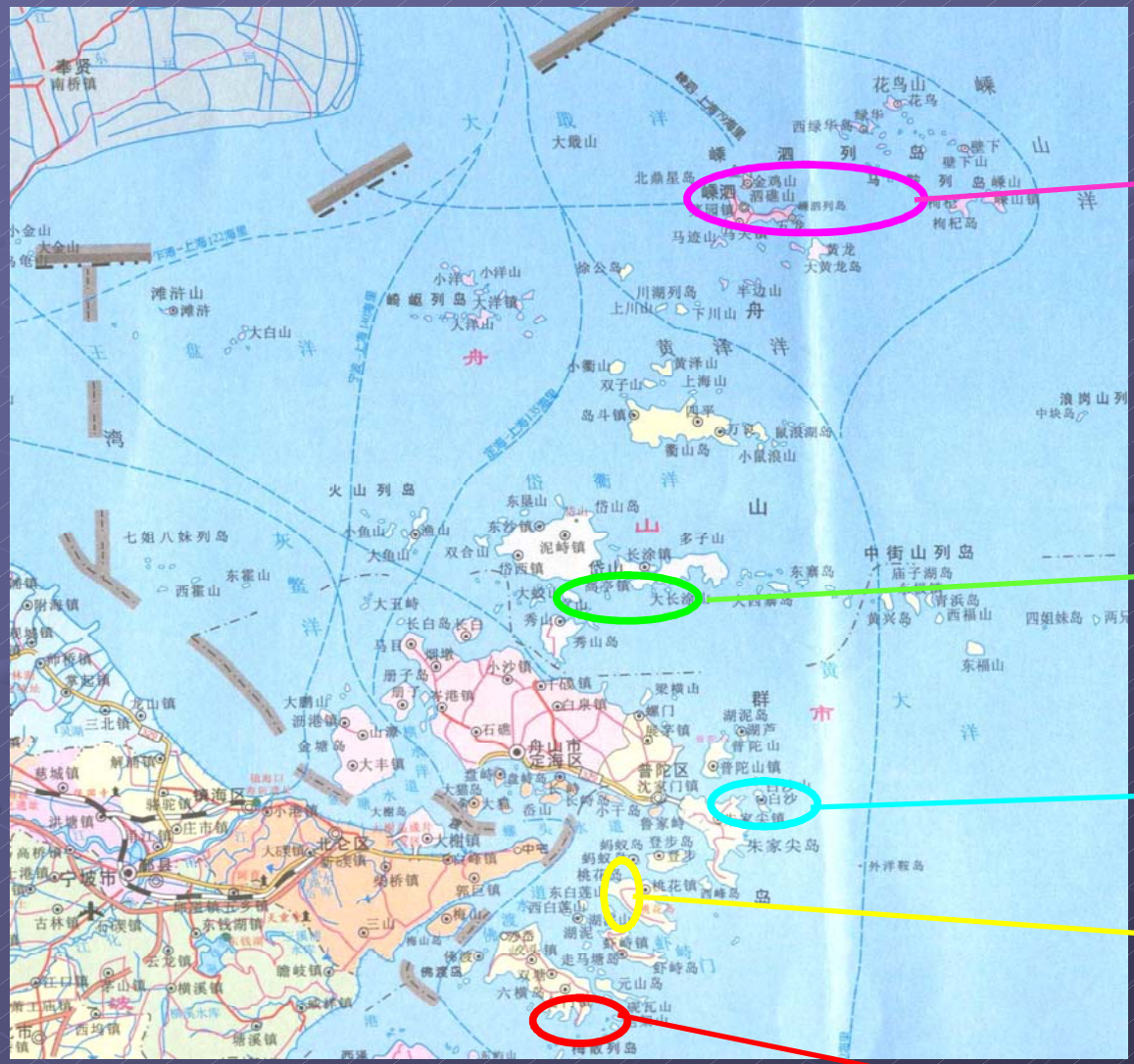
HAB distribution of *procentrum dentum* in May,2004

Photo taken during survey in East China Sea in May,2002

Survey goal

- So in this research variety of shellfish were collected and analyzed for PSP & DSP toxins during spring time through the year 2002 to 2004 in Zhoushan islands. The goal of this survey is to obtain the HAB toxin data in Zhoushan area as well as to value the shellfish contaminate situation in this area and to give the scientific basis for local seafood safety.

Sampling sites in Zhoushan islands



- Survey season:

  3-5 June of 2002, 22-26 May of 2003 and 23-28 April of 2004.

- Investigative items:

  All the samples were first detected for PSP and DSP toxins by mouse bioassay. PSP toxins were also analyzed by HPLC. Special sample was even conducted with more tests in order to find the toxicity source. Totally 41 samples with 20 species were collected during the three year survey.

Materials and Methods

- *Sample colletcting*

  After collecting the shellfish samples, clean the outside of shellfish with fresh sea water and open the shell at once by cutting adductor muscle or breaking the shell. Rinse inside with fresh water to remove sand or other materials. Carefully remove the meat, absorb the surface water with gauze and pick out shell pieces. Finally seal the shellfish meat samples in plastic bags and freeze for toxin analysis.

Mouse bioassay for PSP toxins

Toxicity determination was carried out according to AOAC standard mouse bioassay for PSP toxins. The sample was thawed and homogenized, weigh 100g of well mixed sample into a beaker, add 100ml 0.1M HCL and stir thoroughly, check pH and adjust to pH 2.0-4.0. Heat the sample mixture by water bath and boil for 5 minutes. After it cools to room temperature, detect and adjust pH to 2.0-4.0 again. Let settle until the supernatant is clear enough to be extracted by 5ml injector without blocking the needle. Thick samples are centrifuged to obtain the supernatant. Use male ICR strain mouse with the weight of 17-21g. Mouse Unit (MU) is the toxicity unit for PSP toxins, one mouse unit is refined as the amount of PSP toxin required to kill a 20g mouse within 15 minutes.

Mouse bioassay for DSP toxins

DSP mouse bioassay procedure was conducted according to the method created by Yasumoto. According to the method, the sample of homogenized shellfish meat is extracted with acetone, which is removed by rotary evaporator and the left is further extracted twice by ether. Ether is then also removed by rotary evaporation. The thick residue is re-suspended in 1%Tween 60 solution. A mouse unit is defined as the minimum quantity of toxin required to kill two of three mice in 24h after i.p. injection.

HPLC analysis for PSP toxins

1ml of HCL extracts was centrifuged for 15 minutes at 13,000 g. The supernatant is then passed through a 0.2μm filter and ready for HPLC analysis.

HPLC analysis is adopted post-column derivatization HPLC method by Oshima. Waters HPLC system is used with the following apparatus: a 600E pump, Symmetry RP8 column (3.9×150mm), a 717 auto-sampler, a post-column oxidation system (reaction temperature 65° C), a 2475 fluorescence detector (Ex 330nm, Em 390nm), as well as an Empower software.

HPLC analysis for DSP toxins

The determination of okadaic acid and dinophysistoxin-1 was mainly according to the method by Lawrence et al in 1996. It is extracted with methanol/water, derivatized by 9-chloromethylanthracene (9-CA), cleaned up by dichloromethane and hexane and eluted by acetonitrile on a RP-18 reversed column. Fluorescent detection was observed at 412 nm with 365 nm excitation.

HPLC analysis for TTX

Sample extraction and HPLC system for TTX is similar to PSP. The analysis is adopted HPLC method for TTX by Chen et al used a C18 column(4.0×250mm) with a mobile phase of 2mM heptanesulfonic acid in 0.05mM potassium phosphate buffer, flow rate 0.5ml/min. Oxidation solution used 4N NaOH and eluate 0.5N NaOH. Fluorescent detection was observed at 505 nm with 381nm excitation.

Results

Jiaozhou Bay Monitoring

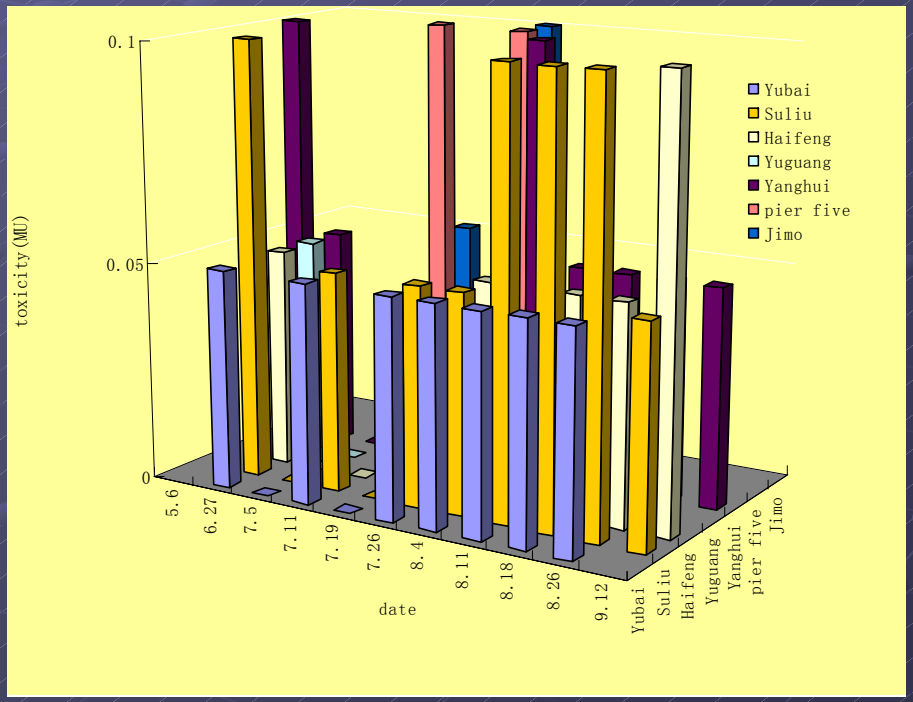
- Phytoplankton monitoring results

Phytoplankton monitoring, used as an early warning of toxic HABs, is carried out with the biotoxin monitoring. During the 3-year investigation, it is found that, in this research area, the potential toxic algea are:

Dinophysis acuminata,
Karenia Mikimotoi,
Alexandrium spp.,
Prorocentrum minimum,
Gynodinium spp. ,
Gynodinium catenella &
Dinophysis fortii.

HAB toxin monitoring results

No shellfish toxicity was found in the year 2001. Two samples were found to contain DSP in April, 2002, with a toxicity of 0.05MU/g. However, DSP was found in most samples in the year of 2003, with the toxicity in the range of 0.05 ~0.1 MU/g. Weekly sampling and analysis were carried out in July and August after DSP was first found in June.



DSP monitoring results in 2003

- HPLC analysis for some of the samples with high toxicity shows that, most samples have no detectable DSP toxins or only a trace, except for one sample collected in July, which contains 54 ng/g of OA. Toxicity detected by mouse bioassay is higher than that by HPLC. The shellfish extracts were cleaned up to remove fatty acid, but the toxicity remained by mouse bioassay. The extracts need to be examined for other DSP toxins, like yessetoxin.
- No PSP toxin was found during the three years of monitoring.

Zhoushan islands investigation

- *Mouse bioassay results*

Shellfish species	Sampling sites	Toxicity (Mu/g)	Growth environment
Mytilus coruscus	Dongji Island, Putuo	<1.7	wild
Mytilus edulis	Gouqi island,Shengsi	0	Shrimp pond
Chlamys farreri	Liuheng Island, Putuo	0	Shrimp pond
Meretrix meretrix	Liuheng Island, Putuo	0	Shrimp pond
Scapharca subcrenata	Liuheng Island, Putuo	0	Shrimp pond
Sinonovacula contrica	Liuheng Island, Putuo	0	Shrimp pond
Tegillarca granosa	Daishan island	0	Shrimp pond
Ruditapes philippinarum	Daishan island	0	Shrimp pond
Sinonovacula contrica	Daishan island	0	Shrimp pond

Table 1. Toxicity of PSP in shellfish collected in Zhoushan Islands (by mouse bioassay, June, 2002)

Shellfish species	Sampling sites	Toxicity(Mu.g^{-1})		Growth environment
		PSP	DSP	
Purpura clavigera	Shengsi Islands	0	0	wild
Rapana venosa	Shengsi Islands	0	0	wild
Mytilus coruscus	Shengsi Islands	0	0	wild
Barbati virescents	Shengsi Islands	<1.7	0	wild
Ostrea crenulifera	Shengsi Islands	0	0	wild
Cultellus attenuatus	Shengsi Islands	0	0	wild
Ruditapes philippinarum	Shengsi Islands	0	0	wild
Mytilus coruscus	Dongji Island, Putuo	0	0	wild
Notoacmea schrencki	Dongji Island, Putuo	<1.7	0.05	wild
Ostrea crenulifera	Liuheng Island, Putuo	0	-	wild
Nassarius sp.	Liuheng Island, Putuo	3.84	<0.05	wild
Babylonia lutosa	Liuheng Island, Putuo	0	0	wild
Ostrea crenulifera	Daishan Island	0	0	wild

Table 2. Toxicity of PSP in shellfish collected in Zhoushan Islands (by mouse bioassay, May, 2003)

Shellfish species	Sampling sites	Toxicity(Mu.g^{-1})		Growth environment
		PSP	DSP	
Babylonia lutosa	Zhujiajian	0	<0.05	wild
Thais bronni	Dongji Island, Putuo	0		wild
Mytilus coruscus	Dongji Island, Putuo	0	0.05	wild
Rapana venosa	Dongji Island, Putuo	0		wild
Notoacmea schrencki	Liuheng Island, Putuo	0	0.05	wild
Monodonta labio	Liuheng Island, Putuo	0	<0.05	wild
Barbati virescents	Liuheng Island, Putuo	0		wild
Thais bronni	Liuheng Island, Putuo	0	<0.05	wild
Chlorostoma custicum	Liuheng Island, Putuo	0		wild
Nassarius siquinjorensis	Liuheng Island, Putuo	8.8		wild
Tegillarca granosa	Liuheng Island, Putuo	0		wild
Mytilus coruscus	Shengsi Islands	0	0.05	wild
Mytilus galloprovineialis	Shengsi Islands	0		wild
Ostrea crenulifera	Shengsi Islands	0		wild
Cultellus attenuatus	Shengsi Islands	0		wild
Ruditapes philippinarum	Shengsi Islands	0		wild
Sinonovacula constricta	Taohua lsland	0		wild
Ostrea crenulifera	Taohua lsland	0		wild
Turritella bacillum	Nanji islands	<1.7	<0.05	wild

Table 3. Toxicity of PSP in shellfish collected in Zhoushan Islands (by mouse bioassay, April, 2004)

- *HPLC analysis for PSP toxins*

  No PSP toxins were found by HPLC analysis in all the samples, especially the *Nassarius* samples which show a high PSP toxicity on mouse.

- *HPLC analysis for TTX*

  Nassarius samples collected in Liuheng island during the spring of 2003 and 2004, which show mouse neural toxicity yet no PSP peaks by HPLC, were further analyzed for TTX by HPLC method. Unfortunately, no positive result was observed.

Conclusions & discussions

- From our three-year monitoring and study on toxic HAB in Jiaozhou Bay, we found that DSP is much more frequent than PSP in the north sea of China. It has been increasing in the recent years. It is necessary to pay more attention to this issue. The results of toxic algae and shellfish toxin monitoring do not always correlate well with each other. It is suggested that phytoplankton monitoring serves best as a tool of early warning.

- The spring investigation of HAB toxins in Zhoushan islands of East China Sea for continuously three years shows that, though there have been frequent red tides happened during spring time in this area recently, the situation of shellfish contamination by HAB toxins is not serious, but increasing.

- The neurotic toxin found in the body of *Nassarius siquinjorensis* in Zhoushan islands is serious and need to be paid great attention to. It has caused 44 poisoning events with 18 human deaths in Zhoushan islands during 1977-2003. Through our survey, it is neither any PSP toxins we know and could test by HPLC, nor TTX. The past recorded paralytic shellfish poisoning events were most identified by mouse bioassay or human symptoms, but through our survey we found that other unknown toxins with similar toxicology as PSP or TTX could have same symptoms on mouse or human. It might be a new derivative of PSP toxin or some other unknown neurotic toxin.

Acknowledgements

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