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Seasonal variation of paralytic and amnesic shellfish toxicities in bivalves and microalgae in Haiphong area, Vietnam

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Abstract — Monitoring survey was conducted to know the distribution and seasonal variation of PSP and ASP toxicities in bivalves and the abundance of toxic microalgae in Haiphong area, Vietnam. Sampling was carried out at the stations in Cat Ba and Do Son every two weeks from February 2002 to March 2004. Low levels of PSP and DA were detected in shellfish samples from both stations, showing seasonal and yearly variation. Toxicity of plankton samples also showed fluctuation, though the clear correlation could not be observed with the shellfish toxicities. HPLC or LC-MSMS analysis provided clear evidence of shellfish contamination with these toxins and indicated the existence of causative microalgae for these toxicities in this area. Several species of *Alexandrium* such as *A. minutum, A. tamiyavanichii, A. ostenfeldii, A. tamarense* were found, though the abundance of them was very low. On the other hand, massive bloom of *Pseudo-nitzschia* occurred in January at both stations. However, these blooms did not always cause the increase of DA level both in plankton and shellfish samples.

Key words: Paralytic shellfish poisoning, Amnesic shellfish poisoning, *Alexandrium*, *Pseudo-nitzschia*, Vietnam, monitoring, Haiphong

Introduction

Contamination of bivalves with shellfish toxins such as paralytic shellfish poison (PSP), diarrheic shellfish poison (DSP), and amnesic shellfish poison (ASP) globally causes severe damage to public health as well as fisheries industry. Causative organisms of shellfish contamination have been shown to be several toxic species of dinoflagellate and diatom. One of the recent problems of shellfish poisoning is the increase in its frequency and intensity as well as the expansion of geographic distribution of causative microalgae (Hallegraeff 1993). It is also true for the southeast Asian countries. Recently, the first PSP incidents including fatal cases were reported from Malaysian waters (Usup 2002) and the occurrence of four species of toxic *Alexandrium* was confirmed from this area (Lim 2002). Human poisoning due to *A. minutum* occurred at Bolinao and Pangasinan in 2003, which was the first record in the history of PSP in Philippines (Bajarias et al. 2003)

In Vietnam, human poisoning by consumption of shellfish has not been recorded so far. However, occurrence of causative species of PSP such as *Alexandrium* has been found in several regions including Haiphong area (Thuoc 2000). Yoshida et al. (2000) first found the toxin production of *A. minutum* from shrimp pond in Quang Ninh. Lam (2004) reported the occurrence of potentially toxic dinoflgellate *A. affine* from Ha Long Bay. On the other hand, Kotaki et al. (2000) found the domoic acid production in *Nitzschia* sp. isolated from a shrimp culture pond in Do Son. These findings suggested the possibility of contamination of marine organisms with toxins and outbreak of human poisoning in these areas. However, the relationship between the occurrence of toxic microalgae and the contamination of toxins in shellfish has been still unclear due to a lack of systematic monitoring system in this area.

In this study, we conducted two years monitoring on shellfish and plankton toxicity along with the abundance of suspected microalgae in coastal waters of Haiphong, Vietnam, to asses the toxins contaminated in shellfish and the possibility of outbreak of shellfish poisoning in future.

Materials and Methods

Phytoplankton and shellfish samples were collected every two weeks at the station (Fig. 1) of Do Son and Cat Ba for two years from February 2002 to March 2004. Phyto-

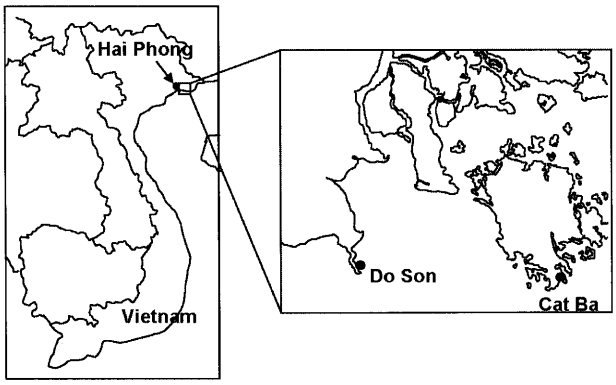

Fig. 1. Map showing sampling sites in Haiphong area, Vietnam.

plankton samples were collected by vertical net (net opening: 20 cm, mesh size: 20 μm) hauling from bottom layer to surface. Seawater samples were also taken with a Van Dorn water sampler from three layers of surface, middle, and bottom. Five liters of the water sample combined were concentrated to 10 ml by filtration with GF/F (Whattman) glass fiber filter. A part (5 ml) of the concentrated sample was fixed with Lugol solution and observed under a light microscope to count cell number of *Alexandrium* and *Pseudo-nitzschia* at genus or species level.

Cultured mussels, *Mytilus edulis* and wild clams, *Meretrix meretrix* were collected at stations of Cat Ba and Do Son, respectively. Plankton collected by the net hauling were recovered on GF/F filter by filtration, suspended in 50% MeOH, sonicated, and centrifuged to obtain toxin extract. Ten grams of digestive gland of the mussels or clams were homogenized with 20 ml of 50% MeOH. After centrifugation, the supernatant was taken and used for toxicity assay by ELISA. Toxin extracts were stored at −20°C until use.

Toxicity of shellfish and plankton was monitored by ELISA for PSP and ASP toxins. Fifty percent MeOH extract was evaporated to dryness and dissolved in certain volume of distilled water. ELISA was carried out according to the methods of Branaa et al. (1999) and Kodama (2003) for DA and PSP analysis with slight modifications, respectively. When relatively high toxicity was detected by ELISA, the samples were also analyzed by HPLC to confirm the existence of toxic components. For PSP toxins, HPLC-fluorescence analysis was carried out according to the method of Oshima (1995). In the case of ASP, DA was analyzed by the HPLC method (Quilliam et al. 1989, Pocklington et al. 1990) with slight modifications. Further confirmation of DA was carried out by LC/MS/MS on an Agilent 1100 LC system (Agilent Technologies) equipped with 2000 quadru-pole MS/MS system (Applied Biosystems). Chromatographic separation was performed using a column of Wakosil Navi 5C–18 (Wako) with a linear gradient system which was run between 0 and 15 min from 0.1% TFA to 100% acetonitrile at flow rate 0.2 ml/min. The electrospray ionization interface was operated in positive mode. The mass spectrometer was operated in Q1 scan.

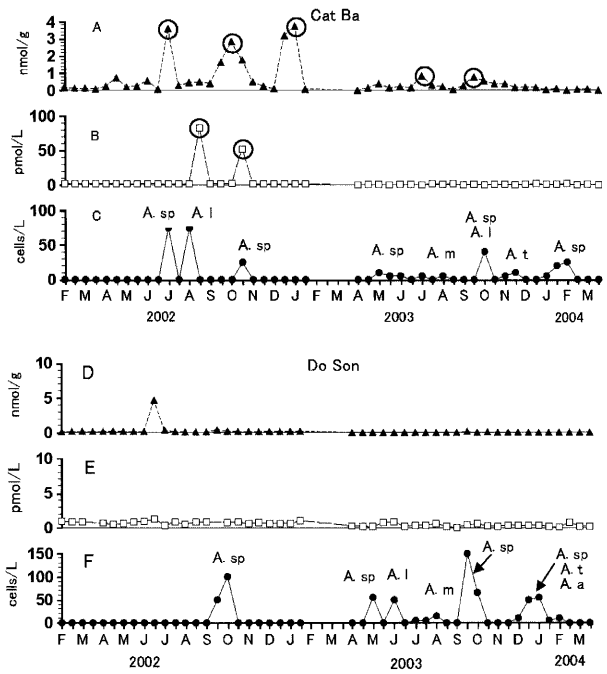

Fig. 2. Variation of PSP toxicity in shellfish digestive glands and plankton samples along with abundance of *Alexandrium* in Haiphong area, Vietnam, from February 2002 to March 2004. A, B, and C: Data for Cat Ba; D, E, and F: Data for Do Son. A and D: Shellfish toxicity (A: green mussels, D: clams); B and E: Toxicity of plankton samples; C and F: Abundance of *Alexandrium* spp. In C and F, species having appeared are also indicated. A.t: *A. tamarense*, A.l: *A. leei*, A.a: *A. affine*, A.m: *A. minutum*, A.sp: unidentified species. Circles in A and B: Samples of which PSP toxins were confirmed by HPLC analysis.

Results and Discussion

Relationship between PSP toxicity in shellfish and abundance of *Alexandrium* spp.

Figure 2 shows the variation of PSP toxicity of shellfish and plankton samples together with the abundance of genus *Alexandrium* at Cat Ba and Do Son. The toxicity of shellfish showed clear seasonal variations, even though the toxicity was much lower than safety level of human consumption. At both stations, it tended to increase from May to August and October to February, suggesting that more than one species of microalgae were involved in the shellfish toxicity. On the other hand, no clear difference in toxicity was recognized between locations or species of shellfish. HPLC analysis of samples with higher toxicities revealed the existence of PSP toxins which were mostly composed of STX, indicating that shellfish at both stations were contaminated with PSP toxins. In the plankton samples, relatively high PSP toxin contents could be detected only from Cat Ba in mid August and late

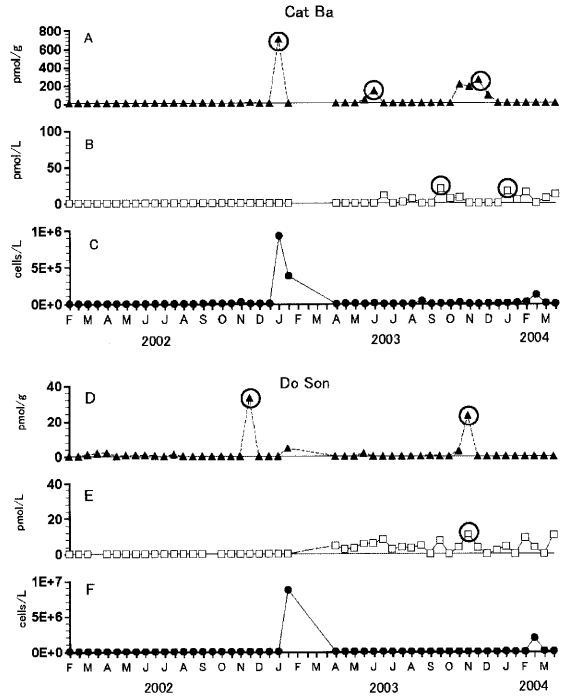

Fig. 3. Variation of ASP toxicity in shellfish digestive glands and plankton samples along with abundance of *Psuedo-nitzschia* in Haiphong area, Vietnam, from February 2002 to March 2004. A, B, and C: Data for Cat Ba; D, E, and F: Data for Do Son. A and D: Shellfish toxicity (A: green mussels, D: clams); B and E: Toxicity of plankton samples; C and F: Abundance of *Psuedo-nitzschia* spp. Circles in A, B, D, and E: Samples of which PSP toxins were confirmed by HPLC analysis and LC-MSMS.

October, 2002. These two samples also showed peaks corresponding to PSP toxins in the HPLC analysis. This strongly suggests the occurrence of causative organisms in this area. Except for these two samples, however, only a low toxicity (less than 2 pmol/l) was detected during the survey period. Several species of *Alexandrium* such as *A. minutum*, *A. tamiyavanichii*, *A. ostenfeldii*, *A. tamarense*, *A. leei*, and *A. affine* were observed at both stations, though the abundance of them was very low. *A. minutum* and *A. leei* appeared at both stations from June to October, whereas *A. tamarese* and *A. affine* occurred at Do Son during December and January. In addition to them, unidentified *Alexandrium* (designated as *Alexandrium* sp. in this study) was also observed to appear. Among these species, maximum cell number was recorded to be about 30 cells/l for *A. minutum*.

The species mentioned above have already been listed to appear in Haiphong area by one of the present authors before this monitoring program (Thuoc 2000). This, together with the present results, suggested that these species of *Alexandrium* are regular members of phytoplankton community in this area, though they do not bloom in high density so far. On the other hand, increase of shellfish toxicity in October, 2002, overlapped with the occurrence of *Alexandrium* sp. and the increase of plankton toxicity at Cat Ba. In addition, several slight increases of shellfish toxicity occurred when some *Alexandrium* species appeared in the ambient seawater. However, it seemed to be difficult to discuss in detail about the association of these *Alexandrium* with the shellfish toxicity since the level of both parameters were too low as mentioned above. At present, all the *Alexandrium* could be included as a candidate of the causative organism for PSP. Further study such as confirmation of toxin production of these species by using culture strain is necessary to identify the causative organisms of shellfish contaminations.

Relationship between DA toxicity in shellfish and abundance of *Pseudo-nitzschia*

Figure 3 shows the variation of DA contents in shellfish and plankton samples along with the abundance of *Pseudo-nitzschia* at Cat Ba and Do Son. DA was detected in several shellfish samples at both stations by ELISA. At Cat Ba, the toxicity peak of clams was observed three times in January, July, and December, 2003, whereas those of green mussels increased in November, 2002 and 2003, in Do Son, though the levels were much lower than those at Cat Ba. In HPLC analysis, the peak corresponding DA was detected in most of shellfish and plankton samples with higher toxicity in ELISA. Furthermore, the existence of DA was confirmed by LC/MS/MS in which the peak with MW of 312 ($M+H^+$) was detected both in shellfish and plankton samples. These results indicate the shellfish contamination with DA and the existence of DA producing organisms in this area also. Production of DA and/or its derivatives have been confirmed in several species of *Pseudo-nitzschia* (Bates et al. 1989, Martin et al. 1990, Garrison et al. 1992) and benthic diatoms such as *Amphora coffeaeformis* (Shimizu et al. 1989) and *Nitzschia navis-varingica* (Lundholm and Moestrup 2000). In this study, we monitored the abundance of genus *Pseudo-nitzschia* to obtain basic information about the mechanisms of DA contamination. Genus *Psuedo-nitzschia* was detected almost through the year. In addition, the appearance was characterized by the massive bloom of this genus from January to February. Maximum abundance reached 928,650 cells/l at Cat Ba in January, 2003, and 8,697,000 cells/l at Do Son in January, 2003, respectively. However, relationship was not so clear not only between the abundance of *Pseudo-nitzschia* and shellfish toxicity, but also between the abundance and plankton toxicities. No significant toxicity was detected in plankton samples at both stations even in samples containing a large number of *Pseudo-nitzschia* cells, suggesting that toxin contents of *Pseudo-nitzschia* are extremely low. On the other hand, increase of clam toxicity at Cat Ba in January, 2003, occurred during a massive bloom of *Pseudo-nitzschia*. This may indicate that *Pseudo-nitzschia* appearing in this area also cause the shellfish contamination. However, except for this period, relationship between the occurrence of *Pseudo-nitzschia* and shellfish toxicity seemed to be com-

plex. Any increase of shellfish toxicity could not be observed at both stations in February, 2004, when *Pseudo-nitzschia* appeared massively in the water column. On the contrary, shellfish toxicity showed peaks during periods when cell density of *Pseudo-nitzschia* was low; *e.g.* in July and November-December at Cat Ba, and November, 2002 and 2003, at Do Son. Kotaki et al. (1996) pointed out that shellfish contamination with DA could not be explained easily by the appearance of *Pseudo-nitzschia* in Ofunato Bay, Japan. It is also true for the area of present study. In order to know the contribution of *Pseudo-nitzschia* to shellfish toxicity, feeding experiments of shellfish by using the algal isolate might be necessary. In the present study, DA was detected not only from green mussels but also from clams which were collected from sediments at Do Son. This result implies that causative species of clams is different from that of green mussels. Kotaki et al. (2000) found DA production of *Nitzschia* sp., which was named as *N. navis-varingica* by Lundhom and Moestrup (2000), isolated from shrimp pond in Do Son. We should pay attention to this species also in the further study.

In this study, we found the contamination of shellfish with PSP and ASP toxins even though the level of them was much lower than the level for human consumption. Though the causative microalgae could not be identified due to the low level of shellfish toxicity, the results of present study suggest the possibility of future outbreak of human poisoning with shellfish poisoning since known causative microalgae occur in the area also. Continuous surveillance might be necessary to know how the change of environmental condition will affect a blooming of toxic microalgae and toxin contamination in shellfish.

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