Dinophysis spp. recorded in the coastal waters of northern Vietnam during 2002–2003

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Abstract — A monitoring on toxins responsible for Diarrhetic Shellfish Poisoning (DSP) was carried out at several culture areas of hard clam (*Meretrix meretrix*) in the northern coast of Vietnam during May 2002 and December 2003. Quantitative observation of *Dinophysis* spp. was made one to four times every month and DSP toxins in the hard clam grown in the area was analyzed monthly using the HPLC method.

Six species of *Dinophysis* were recorded, including *D. caudata, D. fortii, D. miles, D. rotundata, D. mitra* and *D. hastata*. Among them, *D. caudata* was the most common species. Others were not common and their densities were ignorable. *D. caudata* appeared almost all around the year at all sampling sites with density ranged from 0 to more than 3000 cells/l. However, it was more abundant during the warm period, i.e. from late February to late November. For the rest of a year, cells were rarely found. *D. caudata* was recorded in a wide range of temperature (15–34.3°C), but its significant density (over 100 cells/l) was only observed when water temperature exceeded 20°C. *D. caudata* appeared and could reach high density at all salinity in the range of 8-34 PSU. The highest recorded density was 3128 cells/l at 22°C and 8.2 PSU in Thanh Hoa area in February 2003. "Blooms" of *D. caudata*, together with other dominant dinoflagellates, usually coincided with the vanishing period of diatoms.

Okadaic acid (OA) was detected in edible part of clams but at low concentration in all samples. Maximal level was 80 ng OA /100 g, encountered in Thanh Hoa transect during August 2002 and April 2003. DTX 1–4 were not detected in all samples. No significant correlation between OA concentration in clam and the density of *D. caudata* in the water sample was observed, although toxins sometimes peaked the same time with *D. caudata* density. Low concentration of toxins implicates low risk of DSP and explains the absence of poisoning cases in the area so far. However, other shellfishes in the area should also be subjected to toxin monitoring.

Key words: *Dinophysis caudata*, toxic dinoflagellate, DSP toxin, hard clam, Vietnam

Introduction

Some species of *Dinophysis* are known to be sources of toxins causing Diarrhetic Shellfish Poisoning (DSP), which is regularly monitored in Japan, USA and some of European countries. But in Asia most of countries have not set effective monitoring program yet, because of lack of resources. Vietnam has never had any reported case of DSP so far. However, risk of DSP in the country is still uncertain, as the phenomenon may have been confused with other gastrointestinal disorders such as bacterial poisonings by medical doctors. Recently, hard clam (*Meretrix meretrix*) culture become widely operated in the coastal waters of the northern Vietnam and has significant contribution to the local economy. In response to the requirements from the market, the evaluation for DSP risk in these products is required. A study, aiming to record the temporal and spatial variation of *Dinophysis* spp. and the level of DSP toxins in hard clams, was carried out in the coastal waters of the northern part of Vietnam, from Thai Binh to Thanh Hoa provinces, during May 2002–October 2003. This report summaries main results of the study.

Materials and Methods

The sampling was carried out in main culture areas of hard clams in three provinces, Thai Binh, Nam Dinh and Thanh Hoa, which are within the estuarine region of the Red River system (Fig. 1). In each area, a 10 km-transect was set, running rectangular to the coastline. Each transect consisted of four stations: (1) land-based tiger shrimp (*Penaeus monodon*) culture pond; (2) clam farms, about 0.5 km away from the shore; (3,4) two stations about 3 and 10 km away from the shore, corresponding to the depths of about 7–8 m and 12–15 m respectively. Samples were taken at three different depths available, i.e. surface, 5 m and 12 m deep.

Plankton samples were taken one to four times every

month. Qualitative samples were collected by vertical towing using a 20 μm mesh-size net from bottom to surface. Each quantitative sample consisted of 2 litters of seawater taken by a Van-Dorm plastic water sampler. After sedimentation to concentrate to 20 ml, 1 ml aliquot from the concentrated sample was mounted and counted for *D. caudata* and other *Dinophysis* cells using a Sedgewick-Rafter counting chamber under a Nikon E600 epifluorescence microscope. Nutrients in forms of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-} and SiO^{3-} were analyzed twice a month by spectrographic method, using a HACH-DR2010. Temperature and salinity were recorded at the field using a HACH-DR2010 Sension 2.

Hard clam *Meretrix meretrix* grown in the area was collected monthly for DSP toxin analysis. Clam samples were

Fig. 1. Map showing sampling stations for *Dinophysis* spp. monitoring.

instantly preserved in ice after collection from the field. At laboratory, hard clam flesh was subjected to DSP toxin analysis following Lee et al. (1989), and HPLC analysis following Quilliam & Wright (1995).

Results

Six species of *Dinophysis* were recorded, including *D. caudata*, *D. fortii*, *D. miles*, *D. rotundata*, *D. mitra* and *D. hastata* (Fig. 2). Among them, *D. caudata* was the most common species, recorded at all sampling sites and almost throughout the year. Other species were not common and their densities were ignorable.

D. caudata density showed strong temporal variation during the study period in the range of 0 to more than 3000 cells/l. The species was found almost all around the year but it was more abundant during the warm period (late February through late November). In the abundant season the cell density was exceeding 500 cells/l, while rarely encountered in the rest of the year.

D. caudata was commonly found in various types of estuarine waters, from strongly diluted brackish to nearly-oceanic salty waters. Salinity within the range of 8–34 PSU seemed not to be the limiting factor for *D. caudata* distribution. High density could be reached at all salinity within this range. Temperature, in contrast, seemed to limit the occurrence. Though cells could be found in a wide range of temperature (15–34.3°C), significant density, over 100 cells/l, only happened when water temperature exceeded 20°C. The highest recorded density was 3,128 cells/l at 22°C and 8.2
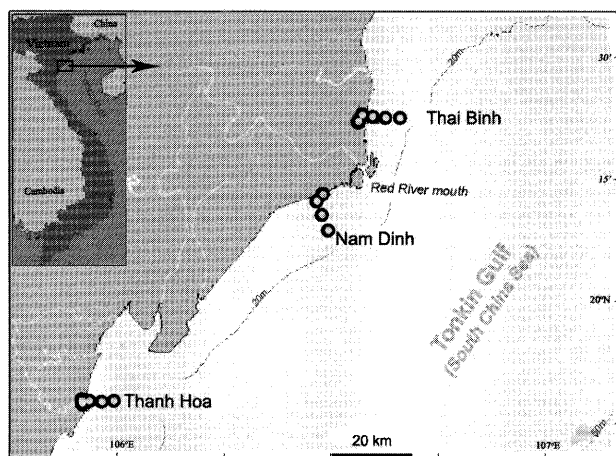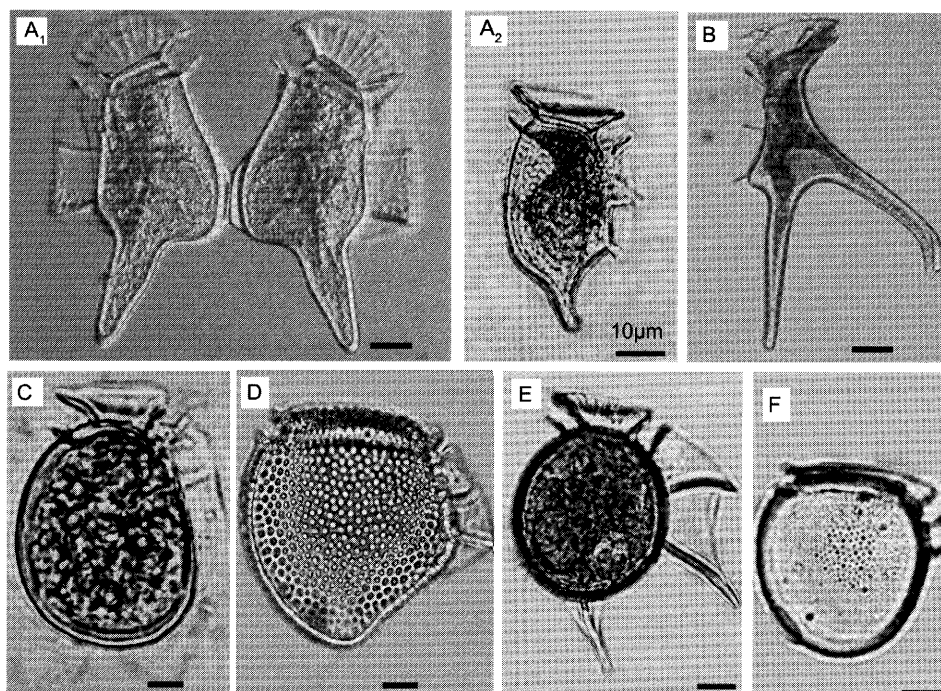

Fig. 2. *Dinophysis* species recorded in the northern Vietnam during 2002–2003.
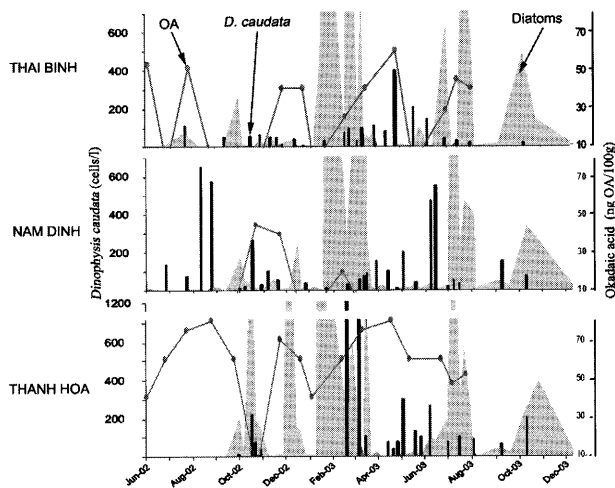
Fig. 3. Temporal variations of average density of *D. caudata* and diatoms in Thai Binh, Nam Dinh and Thanh Hoa transects during June 2002–December 2003.

Fig. 4. HPLC chromatograms of extract of hard clam grown in the study area (A) and DSP toxin standard (B).

psu in a hard clam farm in Thanh Hoa area during February 2003. High density, or "bloom", of *D. caudata*, always occurred together with other dominant dinoflagellates and usually coincided the vanished period of diatom. The replacement was clearer during the early spring, when temperature rose above 20°C.

All other species of *Dinophysis* showed low density all around the year, except *D. rotundata* (up to 400–500 cells/l during 15/7–2/8/2003) and *D. fortii* (up to 700 cells/l in 2/5/2003), both in the Thanh Hoa transect.

Okadaic acid (OA) was detected in clam but not DTX1-4 (Fig. 4). OA concentration was low in all samples. Maximal level was 80 ng OA /100 g, encountered in Thanh Hoa transect during August 2002 and April 2003. No significant correlation between OA concentration in clam and the density of *D. caudata* was observed, although toxins sometimes peaked at the same time with high density of *D. caudata*.

Discussion

Temperature optimum for the occurrence of *D. caudata* was recorded as 20°C in this study. It is in agreement with those recorded in Halong Bay–Vietnam (Larsen and Nguyen 2004), South India (Santhanam and Srinivasan 1996), California–USA (Lechuga-Deveze and Morquecho 1998). From these records, it is speculated whether temperature of around 20°C is the lower limit for *D. caudata* to bloom. This conforms to the distribution pattern of this species, which is, according to Taylor et al. (2004), limited to tropical and subtropical regions. And it, therefore, not surprising that this species is almost absent in the study area—the northern part of Vietnam—during the winter time, when temperature is frequently bellow 20°C.
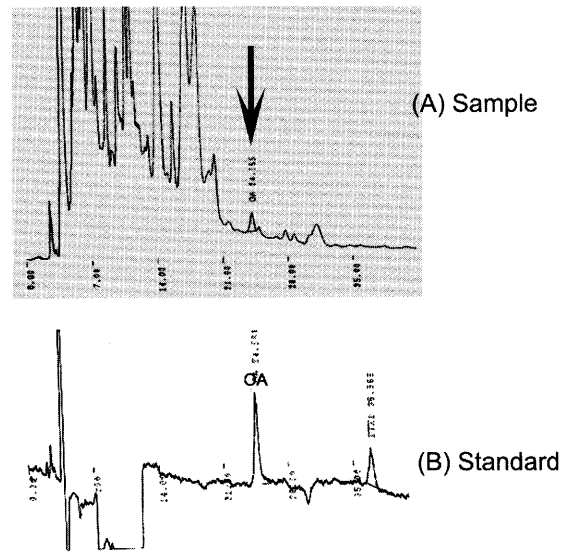
Dinophysis caudata is well known as a common species

in the coastal waters. However, very few studies stated its optimum salinity. So far, Larsen & Nguyen (2004) reported 15–35 psu as the favorite salinity of this species. The high density (over 3,000 cells/l) of this species was found at salinity as low as 8.2 psu, and it indicates that this species is capable to grow well in a wide range of salinity, at least from 8.2 to 35 psu.

Dinoflagellates often bloom in the diminishing period of diatoms, as previously observed by Nakata (1982) and Shimada et al. (1996) in Funka Bay, Japan, and Lechuga-Deveze & Morquecho (1998) in California waters. It shows the competing relationship between diatoms and dinoflagellates, and the competition may play an important role for determining the population level of *D. caudata*. Tsunogai (1979) suggested this relationship was driven by the depletion of dissolved silicate after diatom blooms, and it led to a surplus of nitrogen and phosphorus in the water, enabling dinoflagellates to grow. Roberts et al. (2003) also found high N:Si ratio in waters flavored dinoflagellates, but not diatoms. Our observation supports the Tsunogai's suggestion, as we observed that dissolved silicate dropped to very low concentration after dense blooms (over 10^6 cells/l) of diatoms *Pseudonitzschia* spp. several times (Nguyen, unpublished). Also blooms of diatoms seemed to be strongly dependent on the availability of sunlight. Most cases of dense blooms of diatoms, especially *Pseudo-nitzschia* spp., happened when the sunlight became abundant, after a long period limited sunlight. This is particularly obvious during winter time, when light was limited (Nguyen, unpublished). It seems, then, that phytoplanktons in the sea are well interacted. In which, diatoms play a leading role as they can grow rapidly in response to the change of light condition and then die off because of silicate depletion, which eventually triggers the

growth of dinoflagellates, including *D. caudata.*

Dinophysis species and a benthic dinoflagellates *Prorocentrum lima* are known to be the main sources of DSP toxins. The study area is an estuarine where *P. lima* is rarely found. It is therefore unlikely that the species is the main source of DSP toxins. Among six recorded *Dinophysis* species, five (*D. caudata, D. fortii, D. miles, D. rotundata* and *D. mitra*) have been reported to produce DSP toxins (Marasigan et al. 2001). However, considering its dominant density, *D. caudata* is probably the main source. Recent works carried out by Holmes et al. (1999) and Marasigan et al. (2001) revealed that *D. caudata* cells taken from Singaporean and Philippines waters, respectively, showed to produce okadaic acid, while no DTX-1 toxins was detected. It is thus reasonable that only okadaic acid, but not DTX-1 toxins, were detected in hard clam grown in the area.

In Singaporean waters, where *Dinophysis* density never exceeds 18 cells/l, Holmes et al. (1999) reported consistent low concentration of DSP toxins in green mussels. But the same mussel in the Philippines, where *D. caudata* reached up to several thousands cells in one liter, okadaic acid accumulated up to 1.12 μg/g (Marasigan et al. 2001), which is much higher than the safety level of 0.2 μg/g accepted by most countries. Since shellfish other than hard clam have not been checked for toxins, the real risk of DSP in those shellfish is still uncertain. High density of *D. caudata* encountered in the sampling area, therefore, can not be overlooked, even though DSP toxins concentration in hard clam was low. DSP cases have not been recorded in Vietnam so far, but since DSP can be confused with other types of food poisoning or diseases, those simple statistics are not reliable. Further study on toxin production of *D. caudata* and their accumulation in other shellfish species is thus necessary.

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