Coastal Marine Science 30(1): 130–132, 2006

Domoic acid in a bivalve *Spondylus cruentus* in Nha Trang Bay, Khanh Hoa Province, Vietnam

Dao Viet HA[1*], Yoshinobu TAKATA[2], Shigeru SATO[2], Yasuwo FUKUYO[3] and Masaaki KODAMA[2]

[1] *National Institute of Oceanography, Vietnam, 01 Cau Da, Nha Trang, Vietnam*
** E-mail: tmmp_vnocean@dng.vnn.vn*
[2] *School of Fisheries Sciences, Kitasato University, Sanriku-cho, Ofunato-shi, Iwate 022–0101, Japan*
[3] *Asian Natural Environmental Science Center, The University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan*

Received: 30 September; Accepted: 10 November 2005

Abstract—Recently we have found that domoic acid (DA), a toxin responsible for amnesic shellfish poisoning, is always detected in bivalve species belonging to a genus *Spondylus* randomly collected from various parts of the tropical areas including Vietnam. In Vietnam, 10 species of *Spondylus* are known to grow. Among these species, *S. cruentus* is a commercially valuable species. Domoic acid in *S. cruentus* collected in Nha Trang Bay is analyzed by ELISA and LC/MS/MS. Remarkable individual difference was observed in DA level among 28 specimens of *S. cruentus* collected from the same area at the same time. The DA level in *S. cruentus* apparently showed a seasonal variation. However, the variation of DA content seems to be due to a large individual difference among the specimens. When the specimens were reared in plankton-free conditions, DA level in *S. cruentus* did not decreaseds for 45 days, showing that S. *cruentus* maintains DA for a long period.

Key words: Domoic acid, *Spondylus cruentus*, Nha Trang Bay, Vietnam, ELISA, seasonal variation, depuration

Introduction

Domoic acid (DA) is an excitatory amino acid responsible for amnesic shellfish poisoning (ASP) which was first recognized in Prince Edward Island, Canada in 1987 (Wright et al. 1989). In Canadian case of ASP, *Pseudo-nitzschia multiseries* was identified as a causative phytoplankton (Bates et al. 1989). Since then, several species of *Pseudo-nitzschia* from various parts of the world have been reported to produce DA (Garrison et al. 1992, Lundholm et al. 1994, Martin et al. 1990, Rhodes et al. 1996). Accumulation of DA in bivalves associated with a bloom of *Pseudo-nitzschia* spp. has been also reported from some areas such as Portugal (Vale and Sampayo 2001). However, little is known about DA in tropical waters. Recently, we found that DA is commonly found in bivalve species belonging to a genus *Spondylus* which are randomly collected from various parts of tropical areas including Vietnam (in preparation). In Vietnam, more than 10 species of *Spondylus* are known to grow. Among them, *S. cruentus* is a commercially important species which is consumed in many local restaurants. Thus, it is important from the standpoint of public health to collect information about DA accumulated in *S. cruentus* in Vietnam. In the present study, we analyzed the seasonal change of DA level in *S. cruentus* collected from Nha Trang Bay. Change of DA level in the species which were reared under plankton-free conditions is also analyzed.

Materials and Methods

Materials

Specimens of *S. cruentus* (Fig. 1) were collected by diving at 5–10 m depth in Nha Trang Bay, Khanh Hoa Province, Vietnam (Fig. 2) from December, 2004 to July, 2005. Live specimens were transported to the laboratory of National Institute of Oceanography, Nha Trang, Vietnam. Edible parts of *S. cruentus* specimens were homogenized with 5 volumes of 50% methanol and centrifuged (10,000×*g*, 20 min) according to Quilliam et al. (1989). The supernatants were obtained as crude extracts.

Analysis of domoic acid

Domoic acid was analyzed by enzyme linked immunosorbent assay (ELISA) according to Takata et al. (in preparation). Briefly, the crude aqueous methanolic extract was evaporated to remove methanol, and then the residue was dissolved in phosphate buffered saline (PBS) in a concentration equivalent to 1 g of soft tissue in 10 ml. Each 50 μl of the diluted extract was added to a well of the plates which was coated with DA and blocked with 0.3% gelatin (Bio-Rad, EIA grade), together with 50 μl of the rabbit antiserum against DA at 1 : 3000 dilutions with PBS. After incubation at 37°C for 1 h, the plates were washed 3 times with PBS containing 0.1% Tween 20. One hundred μl of the goat antibody against rabbit immunogloblins labeled with horses

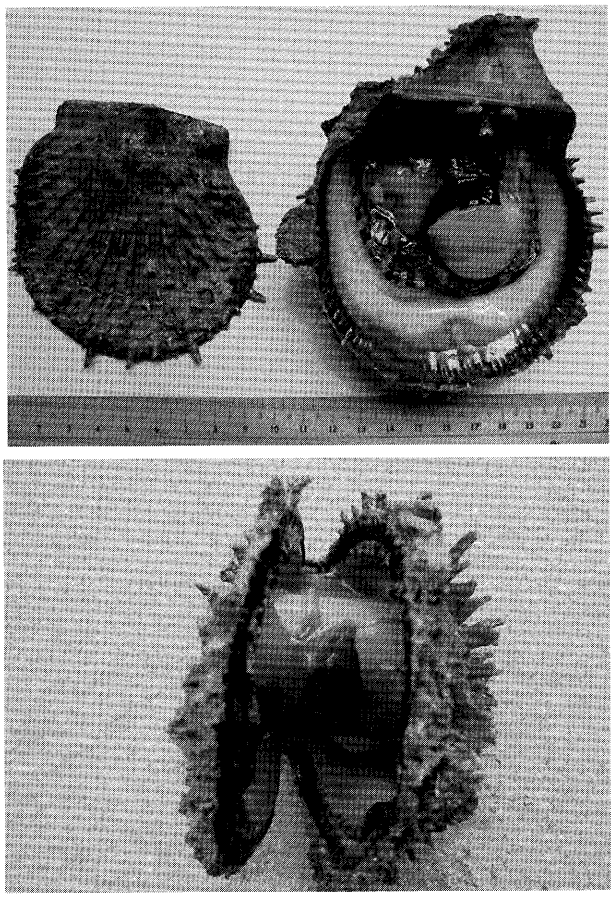

Fig. 1. *Spondylus cruentus.*

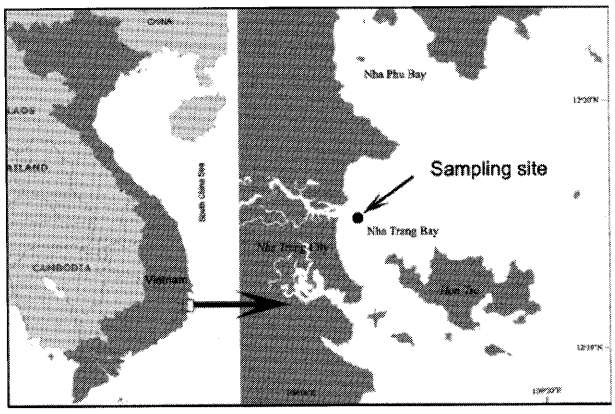

Fig. 2. location of sampling.

radish peroxidase (Dako, P0399, diluted 1000 times with PBS) was added to each well and incubated for 1 h. The anti-DA antibody which binds with DA on the plate was visualized by Fast^{TM} *o*-phenylenediamine dihydrochloride tablet set (OPD, peroxidase substrate, Sigma). Concentration of DA in the sample extract was calculated from the absorbance at 490 nm of samples and DA standard (Bio Vectra) solutions measured by Micro plate reader (Bio-Rad, Model 680). In order to confirm the presence of DA in *S. cruentus*, selected extracts were analyzed by LC/MS/MS (Applied Biosystems, API-2000) according to Takata et al. (in preparation).

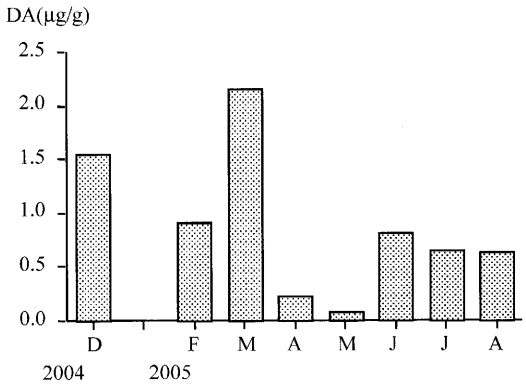

Fig. 3. Seasonal variation of domoic acid level in *Spondylus cruentus.*

Seasonal variation of domoic acid in *S. cruentus*

Three specimens of *S. cruentus* monthly collected from Nha Trang Bay were combined and extracted as described above. Domoic acid level was analyzed by ELISA.

Rearing experiments of *S. cruentus*

Thirty specimens of *S. cruentus* collected in December, 2004 were transferred to an aquarium with Biofiltered-seawater (30°C), and reared until July, 2005. During the period, 3 specimens were taken out biweekly for DA analysis. These specimens were combined, extracted as described above and analyzed by ELISA.

Results and Discussion

Remarkable individual difference was found in DA level among the specimens collected at the same time. In the analysis of DA in 28 specimens of *S. cruentus* collected at May, 2005, all the specimens showed the presence of DA, the level of which ranged from 0.6 to 2.7 μg/g. The mean±standard deviation is 1.37±0.6 μg/g (n=28). In order to confirm the presence of DA in *S. cruentus*, several extracts were selected and further analyzed by LC/MS/MS. In the product ion scan mode of LC/MS/MS, the substance in the *S. cruentus* extracts with the same molecular weight ($[M+H]^+$= 312) and same retention time (10.4 min) as DA standard showed fragment ions such as *m/z* 266, 248, 220 and 161, which are identical to that of the DA standard. These results well coincide with those of ELISA.

Fig. 3 shows the seasonal change in DA levels of *S. cruentus*. The specimens collected at December of 2004 (1.6 μg/g) and March of 2005 (2.2 μg/g) showed higher toxicity while specimens of other months was around 1 μg/g except those collected at March and April which showed significantly lower level. These data apparently show the seasonal

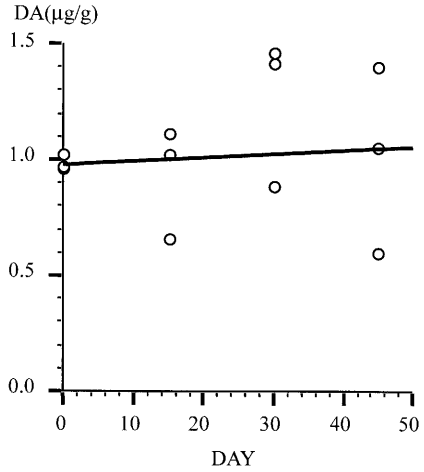

Fig. 4. Change of domoic acid level in *Spondylus cruentus* reared in filtered seawater.

variation of DA level in *S. cruentus*. However, the individual difference in DA of the specimens is very large as described above. Thus, the seasonal variation observed here may be due to a large individual difference of DA level among the specimens

Domoic acid has been detected in a benthic diatom *Nitzschia navis-varingica* which was first collected from a shrimp culture pond in Do Son, Vietnam (Kotaki et al. 2000). Benthic diatoms are hardly considered to be ingested by plankton feeders such as bivalves. However, they are often found in planktonic form in net samples. Actually, *N. navis-varingica* first isolated in Do Son was found in plankton net samples from shrimp culture pond. Further study on DA-producing diatom is necessary in the areas where *S. cruentus* are collected.

Fig. 4 shows the DA level of *S. cruentus* reared in filtered seawater. During 45 days-rearing, the level of DA did not decrease. Domoic acid accumulated in bivalve during a bloom of *Pseudo-nitzschia* spp. is reported to rapidly disappear from bivalve, when the causative species disappear from the environment. Novaczek et al. (1992) reported that more than 10 μg/g of DA accumulated in mussel *Mytilus edulis* disappears within 5 days. These facts show that *S. cruentus* maintains DA for a long period.

Recently, we found that DA is always detected in bivalve species belonging to a genus *Spondylus* which are collected randomly from various parts of tropical areas, while no DA is detected in other species of bivalves collected from the same areas (in preparation). These facts suggest that the origin of DA in *Spondylus* spp. is unknown plankton species with a low level of DA. *Spondylus* spp. seem to accumulate effectively a low level of DA in environmental water. The present result that *S. cruentus* maintains accumulated DA for a long period well supports our previous results.

References

Bates, S. S., Bird, C. J., de Freitas, A. S. W., Foxall, R., Gilgan, M., Hanic, L. A., Johnson, G. R., McCulloch, A. W., Odense, P., Pocklington, R., Quilliam, M. A., Sim, P. G., Smith, J. C., Subba Rao, D. V., Todd, E. C. D., Walter, J. A. and Wright, J. L. C. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish. Aquat. Sci. 46: 1203–1215.

Garrison, D. L., Conrad, S. M., Eilers, P. P. and Waldron, E. M. 1992. Confirmation of domoic acid production by *Pseudonitzschia australis* (Bacillariophyceae) cultures. J. Phycol. 28: 604–607.

Kotaki, Y., Koike, K., Yoshida, M., Thuoc, C. V., Huyen, N. T. M., Hoi, N. C., Fukuyo, Y. and Kodama, M. 2000. Domoic acid production in *Nitzschia* sp. (Bacillariophyceae) isolated from a shrimp-culture pond in Do Son, Vietnam. J. Phycol. 36: 1057–1060.

Lundholm, N., Skov, J., Pocklington, R. and Moestrup, Ø. 1994. Domoic acid, the toxic amino acid responsible for amnesic shellfish poisoning, now in *Pseudonitzschia seriata* (Bacillariophyceae) in Europe. Phycologia 33: 475–478.

Martin, J. L., Haya, K., Burridge, L. E. and Wildish, D. J. 1990. *Nitzschia pseudodelicatissima*—a source of domoic acid in the Bay of Fundy, eastern Canada. Mar. Ecol. Prog. Ser. 67: 177–182.

Novaczek, I., Madhyastha, M. S., Ablett, R. F., Donald, A., Johnson, G., Nijjar, M. S. and Sims, D. E. 1992. Depuration of domoic acid from blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49: 312–318.

Quilliam, M. A., Sim, P. G., McCulloch, A. W. and McInnes, A. G. 1989. High-performance liquid chromatography of domoic acid, a marine neurotoxin, with application to shellfish and plankton. Intern. J. Environ. Anal. Chem. 36: 139–154.

Rhodes, L., White, D., Syhre, M. and Atkinson, M. 1996. *Pseudo-nitzschia* species isolated from New Zealand coastal waters: domoic acid production *in vitro* and links with shellfish toxicity. *In* Harmful and Toxic Algal Blooms. Yasumoto, T., Oshima, Y. and Fukuyo, Y. (eds.). pp. 155–158, Intergovernmental Oceanographic Commission of UNESCO, Paris.

Vale, P. and Sampayo, M. A. M. 2001. Domoic acid in Portuguese shellfish and fish. Toxicon 39: 893–904.

Wright, J. L. C., Boyd, R. K., de Freitas, A. S. W., Falk, M., Foxall, R. A., Jamieson, W. D., Laycock, M. V., McCulloch, A. W., McInnes, A. G., Odense, P., Pathak, P., Quilliam, M. A., Ragan, M. A., Sim, P. G., Thibault, P., Walter, J. A., Gilgan, M., Richard, D. J. A. and Dewar, D. 1989. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem. 67: 481–490.