

## 织纹螺体内的河豚毒素及其衍生物

张农<sup>\*</sup>, 刘海新, 苏捷, 叶孙忠, 李庐峰

福建省水产研究所水产品加工研究室 厦门 361012

**摘要:** 对2002年福建省引起食物中毒的织纹螺毒素进行研究。高效液相色谱-质谱联用(liquid chromatography/mass, LC/MS)的分析结果显示 织纹螺毒素的主要成分是河豚毒素类毒素(tetrodotoxin, TTX), 另外还含有4-*epi* TTX、anhydroTTX、deoxyTTX等成分。生物法对织纹螺毒素的毒力测定结果表明: 所收集的26个织纹螺样品中15个检测出毒性, 毒素的毒力在5.7~47.3 MU·g<sup>-1</sup>之间。毒力最高的织纹螺样品2003年5月1日来自福安, 主要由半褶织纹螺和另一未定种组成。

**关键词:** 毒性; 河豚毒素; 织纹螺

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## Tetrodotoxin and Its Derivatives in Gastropods (*Nassarius*)

Zhang Nong<sup>\*</sup>, Liu Haixin, Su Jie, Ye Sunzhong, Li Lufeng

Fisheries Processing Research Department, Fisheries Research Institute of Fujian, Xiamen 361012, China

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**Abstract:** Toxins in *Nassarius* gastropods during food intoxication event in Fujian Province in 2002 were studied. The toxin extracted from the gastropods was analyzed by high-performance liquid chromatography/mass spectrometry, and the result indicated that the toxin consisted of tetrodotoxin (TTX) and its derivatives, i. e., 4-*epi* TTX, deoxyTTX, as well as anhydroTTX. The toxic equivalent of the toxins was also detected by the bioassay approach, and the results indicated that 15 gastropods were confirmed to be positive among 26 samples, the toxicity in samples ranged from 5.7 to 47.3 mouse units per gram (MU·g<sup>-1</sup>). The gastropods with highest toxicity was collected from Fuán on May 1, 2003, which were consisted of two species, i. e., *Nassarius semiplicatus* and a species not yet documented.

**Keywords:** toxicity; tetrodotoxin; *Nassarius*

Gastropods (*Nassarius*) are common shells mostly distributed in the southeast part of Asia. More than 50 incidents have happened by eating gastropods since 1977<sup>[1]</sup>. In April and May of 2002, more than 60 people were poisoned (Table 1) in Xiapu, Ningde, Putian, Xiamen and Luoyuan of Fujian Province, China (Fig. 1)<sup>[1]</sup>. The victims experienced multiple symptoms that included paralysis of the lips, tongue and extremities. Victims also reported nausea, vomiting, diarrhea, or coma. Three of

them died and the others recovered after two weeks.

As we known, food poisoning incidents due to eating puffer fish that contains TTX have frequently occurred in Japan<sup>[2]</sup> and in China<sup>[3]</sup>. This toxin has also been detected in marine gastropods. The first incident of a gastropod contaminated with TTX was reported in Japan in 1979<sup>[4]</sup>. Similar incident of gastropods contaminated with TTX and PSP also happened in Taiwan<sup>[5]</sup>. In this paper, gastropods were collected in Fujian Province

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作者简介: 张农(1968-)男, 博士, 副研究员, 主要从事水产品加工和质量安全的研究;

\* 通讯作者( Corresponding author), E-mail: zn@fjse.ac.cn

and were analyzed for their toxicity in order to identify toxins and their derivatives in the gastropods.

Table 1 Gastropod poisoning incidents in Fujian Province in 2002

| Date        | Places  | Number of victim | Number of deaths |
|-------------|---------|------------------|------------------|
| Apr. 30     | Xiapu   | 3                | 1                |
| May 4       | Ningde  | 3                | 1                |
| May 7 ~ 11  | Putian  | 24               | 0                |
| May 19 ~ 20 | Xiamen  | 30               | 0                |
| May 21      | Luoyuan | 4                | 1                |
|             | Total   | 64               | 3                |

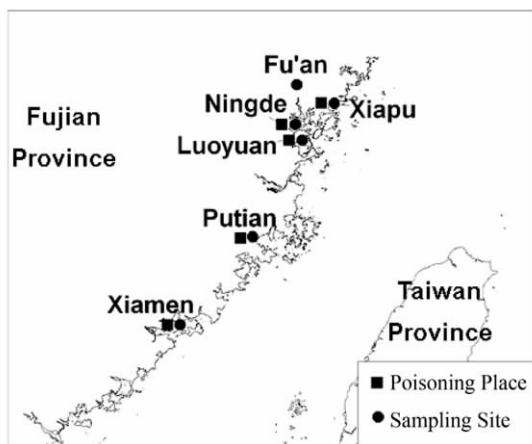


Fig. 1 Poisoning places and sampling sites in Fujian of China

## 1 Materials and methods

### 1.1 Materials

Gastropods were collected from the markets of Xiapu, Ningde, Putian, Xiamen, Luoyuan and Fu'an in Fujian Province from April to May of 2003 (Fig. 1), and stored at 30°C before detection. The shells were removed and the edible parts of the specimens (including digestive gland) were collected. The standard of TTX, 4-*epi* TTX, deoxyTTX, and anhydroTTX were provided by faculty of fisheries, Nagasaki University, Japan.

### 1.2 Toxicity detection by bioassay

The edible part of specimen was weighed and homogenized with two volumes of 0.1% (v/v) acetic acid and heated in boiling water for 10 min then centrifuged. The resulting supernatant was used for toxicity assay. The assay was performed according to Japanese standard method for TTX analysis<sup>[6]</sup>. Shortly, the supernatant was intraperitoneal injected into male mice of the KM strain (body weight 18 ~ 20 g). Using the me-

dian value of mice's death times, the toxicity of specimen can be calculated and the result was expressed as  $\text{MU} \cdot \text{g}^{-1}$ . One MU is defined as the amount of toxin required to kill a mouse in 30 min after injection.

### 1.3 Toxin and its derivatives analyzed by liquid chromatography/mass spectrometry (LC/MS)

The extraction supernatant described above was filtered through a 0.45  $\mu\text{m}$  PVDF membrane (Millipore), and subjected to LC/MS (Waters Breeze) equipped with a ZSpray<sup>TM</sup> MS 2000 detector and a Puresil C18 (4.6  $\times$  250 mm, Waters) column. For LC conditions, 30  $\text{mmol} \cdot \text{L}^{-1}$  heptafluorobutyric acid in 1  $\text{mmol} \cdot \text{L}^{-1}$  ammonium acetate buffer (pH 5.0) was used as mobile phase, and at flow rate of 1.0  $\text{mL} \cdot \text{min}^{-1}$ . About 20% of the eluate was introduced via a splitter into the ion source of MS, and ionized by a positive mode of electrospray ionization (ESI) with desolvation temperature at 350°C, source block temperature at 120°C, and cone voltage at 50 kV. The ionized molecules were monitored through a MassLynx<sup>TM</sup> NT operating system. The result was identified by retention time and  $m/z$  comparing standard substance.

## 2 Results

### 2.1 Toxicity by bioassay

Total twenty-six specimens were tested by bioassay method and the results were shown in Table 2. Among 26 samples, 15 were confirmed to be positive, with a frequency of 57.7%. The toxicity equivalent ranges were 7.0 ~ 21.7  $\text{MU} \cdot \text{g}^{-1}$ , 8.1 ~ 47.3  $\text{MU} \cdot \text{g}^{-1}$  and 5.7 ~ 7.6  $\text{MU} \cdot \text{g}^{-1}$ , for samples from Ningde, Fu'an, and Xiamen, respectively. Samples from Putian, Luoyuan and Xiapu were assayed to be negative or the toxic equivalent were less than 2.0  $\text{MU} \cdot \text{g}^{-1}$  (data not shown). The highest toxicity

Table 2 Toxicity of poisonous gastropods collected in Fujian Province in 2003

| Ningde |  | Fu'an  |  | Xiamen |  |
|--------|--|--------|--|--------|--|
| Date   | Toxicity<br>/( $\text{MU} \cdot \text{g}^{-1}$ ) | Date   | Toxicity<br>/( $\text{MU} \cdot \text{g}^{-1}$ ) | Date   | Toxicity<br>/( $\text{MU} \cdot \text{g}^{-1}$ ) |
| Apr 26 | 21.7   | May 1  | 47.3   | May 6  | 7.6  |
| May 6  | 17.2   | May 25 | 39.3   | May 7  | 6.3  |
| May 7  | 10.9   | May 9  | 9.4  | May 8  | 5.8  |
| May 8  | 7.0  | May 14 | 24.6   | May 14 | 5.7  |
| May 9  | 15.1   | May 15 | 8.5  |        |  |
|        |  | May 16 | 8.1  |        |  |

was  $47.3 \text{ MU} \cdot \text{g}^{-1}$  observed in samples collected from Fu'an (a place near Xiapu) on May 1, 2003.

## 2.2 Toxin and its derivatives

Sample collected from Fu'an on May 1, 2003 was further analyzed by LC/MS, and the results were shown

in Fig. 2 and Fig. 3. A high peak with retention time of 7.34 min, and a  $m/z$  value of 320 was identified. The retention time of the peak was similar to that of the standard TTX (M + H). Peaks occurred at  $m/z$  320, 302 and 304, which were identified as 4-*epi* TTX, deoxyTTX and

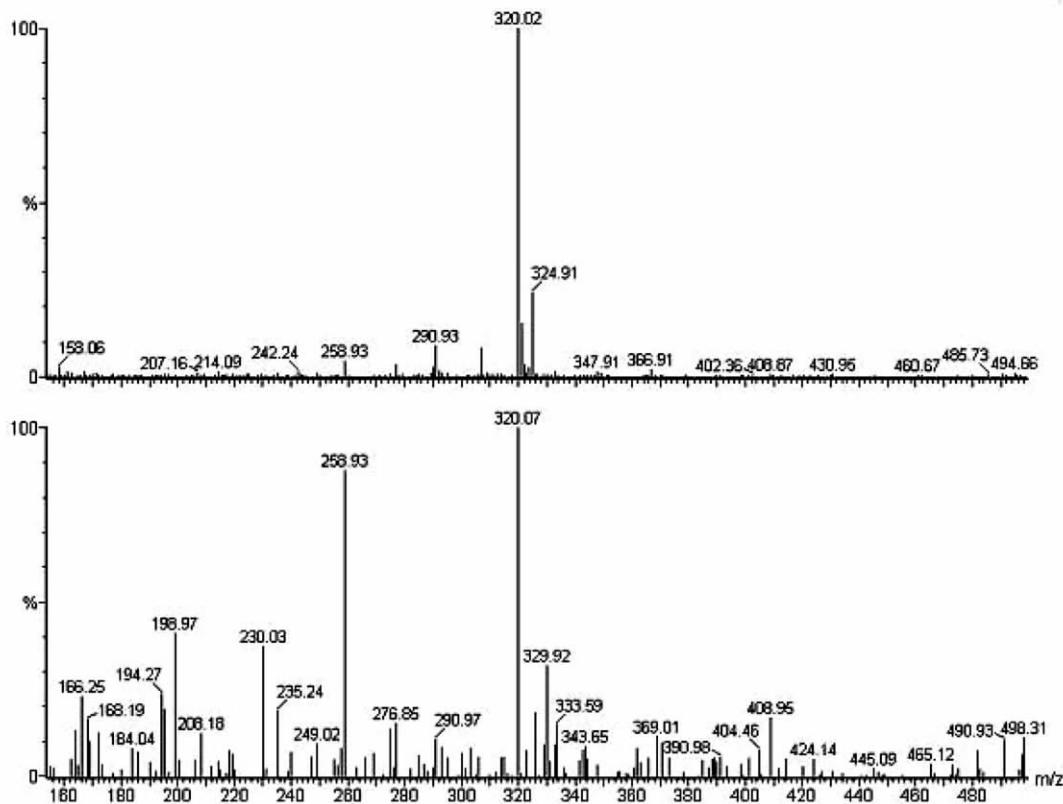


Fig. 2 Mass spectra of electrospray ionization of TTX standard (upper) and gastropod of Fu'an (lower)

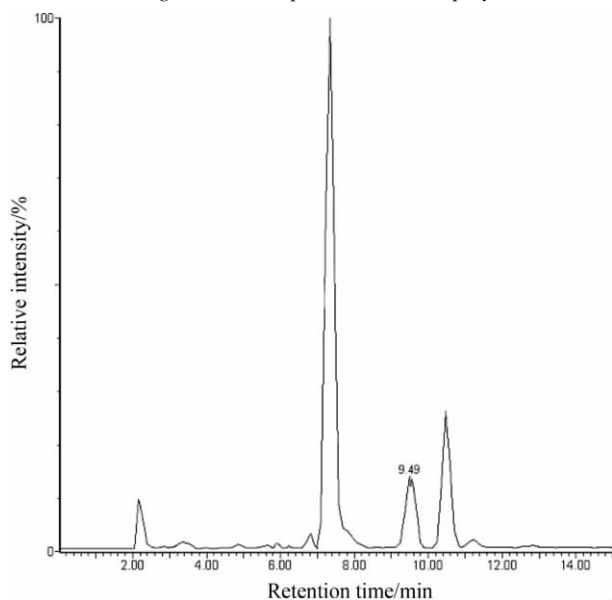


Fig. 3 Mass chromatograms complex of  $m/z$  320, 302 and 304 about gastropod toxin

anhydroTTX, respectively. Therefore, we get the conclusion that the toxin of gastropods *Nassarius* was mainly TTX and its derivations, containing 4-*epi* TTX, deoxyTTX and anhydroTTX.

The ion intensity of the base peak (100% relative intensity) was indicated. Chromatographic conditions are as follows: Puresil C18 ( $4.6 \times 250 \text{ mm}$ , Waters), 30 mM heptafluorobutyric acid in 1mM ammonium acetate (pH 5.0), flow rate:  $1.0 \text{ mL} \cdot \text{min}^{-1}$ .

## 2.3 Characterization of the gastropods species

The gastropods collected from Fu'an on May 1, 2003 were characterized to be consists of two species. One is *Nassarius semiplicatus* (Fig. 4a) with characteristics of a solid and heavy shell; body whorl smooth and glossy, uniformly brown or light brown in color, with two

straw-yellow bars; remainder whorls sculptured with moderately thick axial ribs which form nodules at the suture; outer lip thickened, reflected outwards, with 6 ~ 12 teeth internally, columella calloused, with small denticulate internally, uniform cream or white in color; habitat in the sand and mud flats, from intertidal down to several decadal meters. The other one was not yet well documen-

ted (*Nassarius* sp. Fig. 4b). Its characteristics are shell moderately light in weight, early spire whorls axially and spirally ribbed, uniformly light brown to yellow in color; whole surface sculptured with moderately thick axial ribs; outer lip thin and smooth, without varix and denticles; habitat in the sand and mud flats, similar with *semiplicatus*, from intertidal down to several decadal meters.

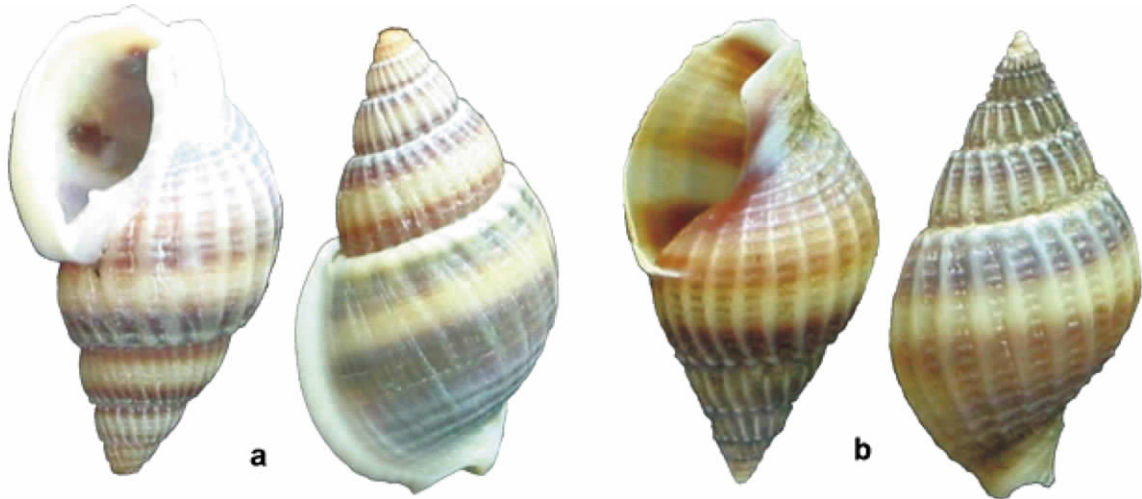


Fig. 4 Photographs of gastropods of *Nassarius semiplicatus* (a) and (b) *Nassarius* sp.

### 3 Discussion

The present results revealed that the toxic equivalent ranges from  $5.7 \text{ MU g}^{-1}$  to  $47.3 \text{ MU} \cdot \text{g}^{-1}$  was lower than that reported in Taiwan<sup>[7]</sup>. Thus, the gastropods in Fujian Province contain moderate amounts of toxin. Ingested gastropods meat by one person is generally less than 100 g at one time. Accordingly, the maximum of ingested TTX is less than 4 730 MU which is small than the minimum lethal dosage of TTX 10 000 MU<sup>[8]</sup>. Thus, it is presumed that the toxicity of gastropods led to food poisoning incidents in 2002 was more than  $100 \text{ MU} \cdot \text{g}^{-1}$ , and was higher than that used in the present study which was collected in 2003. It is probably that the toxin content in gastropods had seasonal properties.

It has been reported that the toxin of gastropods in China was paralytic shellfish poisoning (PSP)<sup>[9]</sup>, probably resulted from the red tide<sup>[10]</sup>. However, Chen et al.<sup>[11]</sup> indicated that the toxin of *Nassarius* was TTX. In our present study, no PSP was identified in gastropods. The composition of toxins in gastropod in Taiwan were identified to be TTX, anhTTX and Gon-

yautoxin GTX1-4<sup>[5]</sup>, which were different from TTX, 4-*epi* TTX, deoxyTTX and anhydroTTX identified in the samples of Fujian Province. Therefore, the compositions of toxin in gastropods varied with regions.

No toxin in the gastropods could be detected in samples from Putian, Luoyuan and Xiapu. However there were *Nassarius* gastropods food poisoning incidents reported in Putian and Luoyuan in May 2002. It was presumed that the frequency of our sampling should be further strengthened in future.

*Nassarius* gastropods are carnivorous animals which usually inhabit in sandy or slimy coastal waters. We presume that *Nassarius* gastropods might accumulate TTX or related substances from food chain in their residence areas. It was reported that TTX-containing animals might absorb and accumulate TTX and some substances produced by bacterium<sup>[12]</sup>. To clarify the source of toxin, animals inhabited near gastropods should be detected for further research.

Reference:

[1] 张农, 刘海新, 苏捷, 等. 织纹螺及其毒性[J]. 中

- 国水产, 2007, 376(3): 72-73
- Zhang N, Liu H X, Su J, et al. The *Nassarius* and toxicity [J]. China Fisheries, 2007, 376(3): 72-73 (in Chinese)
- [2] Nogichi T, Ebesu J S M. Puffer poisoning: Epidemiology and treatment [J]. Journal of Toxicology Toxin Reviews, 2001, 20(1): 1-10
- [3] 蒋骏, 李玉萍, 董林. 河豚中毒 80 例分析 [J]. 中国现代医生, 2007, 45(21): 156
- Jiang J, Li Y P, Dong L. Analysis about eighty Fugu poisoning [J]. China Modern Doctor, 2007, 45(21): 156 (in Chinese)
- [4] Narita H, Noguchi T, Maruyama J, et al. Occurrence of tetrodotoxin in a trumpet shell "boshubora" *Charonia sauliae* [J]. Bulletin of the Japanese Society of Scientific Fisheries, 1981, 47(7): 935-941
- [5] Hwang D F, Cheng C A, Jeng S S. Gonyautoxin-3 as a minor toxin in the gastropod *Niotha clathrata* in Taiwan [J]. Toxicon, 1994, 32(12): 1573-1579
- [6] Kawabata T. Food Hygiene Examination Manual [M]. Tokyo: Japan Food Hygiene Association, 1978
- [7] Hwang D F, Shiu Y C, Hwang P A, et al. Tetrodotoxin in gastropods (snails) implicated in food poisoning in northern Taiwan [J]. Journal of Food Protection, 2002, 65(8): 1341-1344
- [8] 张农, 苏捷, 刘海新, 等. 我国东南沿海地区织纹螺科的种类与毒性 [J]. 生态毒理学报, 2009, 4(2): 249-254
- Zhang N, Su J, Liu H X, et al. The Species and toxicities of *Nassariidae* collected from the coast of Southeast China Sea [J]. Asian Journal of Ecotoxicology, 2009, 4(2), 249-254 (in Chinese)
- [9] 黄曙光. 一起织纹螺引起麻痹性贝类中毒的调查分析 [J]. 实用预防医学, 2003, 10(1): 85-86
- Huang S G. Investigation and analysis in shell *Nassarius* poisoning due to PSP [J]. Practical Preventive Medicine, 2003, 10(1): 85-86 (in Chinese)
- [10] 刘少娟, 陈伟伟, 林健, 等. 毒素污染的红带织纹螺小鼠毒力检测 [J]. 海峡预防医学杂志, 2003, 9(2): 46
- Liu S J, Chen W W, Lin J, et al. The testing of toxin polluted *Nassarius* using mouse [J]. Strait Journal of Preventive Medicine, 2003, 9(2): 46 (in Chinese)
- [11] 陈人强, 李书龙, 关晓理, 等. 食用织纹螺引起的河鲀毒素中毒调查分析 [J]. 中国卫生检验杂志, 2001, 11(2): 251
- Chen R Q, Li S L, Guang X L, et al. Investigation and analysis in TTX poisoning about ingesting *Nassarius* [J]. Chinese Journal of Health Laboratory Technology, 2001, 11(2): 251 (in Chinese)
- [12] Nogichi T, Hwang D F, Arakawa O, et al. *Vibrio alginolyticus*, a tetrodotoxin-producing bacterium, in the intestines of the fish *Fugu vermicularis* [J]. Marine Biology 1987, 94(4): 625-630 ◆