Coastal Marine Science 35(1): 1–6, 2012

High individual variation in the toxicity of three species of marine puffer in Vietnam

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Received 5 December 2009; accepted 8 September 2011

Abstract—In Vietnam, several poisonings due to consumption of marine puffers have been reported every year for a long time, even recently. In most cases, the causative species were those commonly found in Vietnam such as *Torquigener gloerfelti*, *Takifugu oblongus* and *Lagocephalus sceleratus*. In this study, the toxicity of various organs (skin, muscle, intestine, liver and reproductive organ) was analyzed by HPLC for the specimens of three species mentioned above collected from the Khanh Hoa coast in 2007. High variation was observed in the toxicity of the organs of each species. The reproductive organ and the liver showed the highest toxicity, in terms of high average toxicity as well as large toxicity range: the toxicity of the reproductive organ of *T. gloerfelti* was 123.2±240.6 MU g^{-1}, n=30; that of the liver of *T. oblongus* was 171±239.3 MU g^{-1}, n=30. In addition, a significant level of toxin was often found in their muscle. Especially, the muscle of *L. sceleratus* often showed quite high toxicity (58.7±62.3 MU g^{-1}, n=12) which was dangerous for human consumption. 83% of the specimens of this species showed the toxicity higher than safety consumption level suggested in Japan (10 MU g^{-1}). These results show that these puffer species are inappropriate for food because of the contamination of high level of toxin, though some nontoxic or weakly toxic specimens are included.

Key words: toxic puffer, *Torquigener gloerfelti*, *Takifugu oblongus*, *Lagocephalus sceleratus*, tetrodotoxin

Introduction

In the last decade, the poisoning caused by consumption of puffer accounted for 15.1% of all the food poisonings in Vietnam. The estimated number of the victims was 737 peoples with 127 mortalities during 1999–2003. It was most remarkable that puffer poisoning scored high, and was characterized by high mortalities. The mortalities by puffer poisoning accounted for 17.2% of puffer poisonings and for 42.9% of total mortalities by food poisonings (Tran et al. 2005). Marine puffers were usually not used as food materials. However, sometimes people used puffers as materials for fish sauce together with other small fish. People believed that the toxin disappeared during fermentation process of fish sauce.

Among more than 40 marine puffer species found in Vietnamese coastal water, some of them were considered as causative species for food poisoning in Vietnam such as *Torquigener gloerfelti*, *Takifugu oblongus*, *Lagocephalus lunaris*, *L. sceleratus*, and *L. suezensis* (Vo et al. 2004). Dangerously, some considerably highly toxic species such as *L. lunaris* (Dao unpubl.) were listed as economically important species in Vietnam because of their high catch amount (100–200 t yr^{-1}) (Nguyen 1999). To keep seafood safety, puffer fish collected in Vietnam had to be examined systematically. This study presents toxicity data on three common puffer species found in Vietnam.

Materials and Methods

Specimens of puffers

Three common species of puffers, *T. oblongus*, *T. gloerfelti* and *L. sceleratus* (Fig. 1) were collected from Khanh Hoa coast (Fig. 2) in 2007 (Table 1). The specimens were immediately carried to the Laboratory of Biochemistry Department, Institute of Oceanography, Nha Trang, Vietnam in ice-cooled condition.

Extraction and HPLC analysis for individual toxicity variation

Puffer specimens were dissected to skin, muscle, liver, intestine and reproductive organ (ovary or testis). Each organ from each specimen was separately homogenized and extracted with equal volume of 1% acetic acid, and boiled for 5 min. After cooled down to room temperature, the homogenate was messed up to twice volume of the organ with 1% acetic acid. Then the mixture was centrifuged to get the extract. One ml of the extract is equivalent to 0.5 g of organ. These extracts were analyzed by fluorometric HPLC analyzer

for tetrodotoxin (TTX) (Yotsu et al. 1989) and paralytic shellfish poisoing (PSP) toxins (Oshima 1995). Authentic standard of TTXs (TTX, 4-*epi*TTX and 4,6-*anh*TTX mixture) was prepared from the toxic liver of Japanese puffer *Takifugu paradalis* (Kodama et al. 1984). Standard PSP toxins (N-sulfocarbamoyl-11-hydroxysulphate toxin [C]1+C2 mixture, gonyautoxin [GTX]1-4 mixture, saxitoxin [STX], dcSTX and neoSTX mixture) were a gift from Dr. Oshima of Tohoku University. The toxicity of PSP toxins was calculated using specific toxicity of each toxin components (Oshima 1995). The toxicity of TTX was also calculated based on the specific toxicity of each TTX component (Nakamura and Yasumoto 1985).

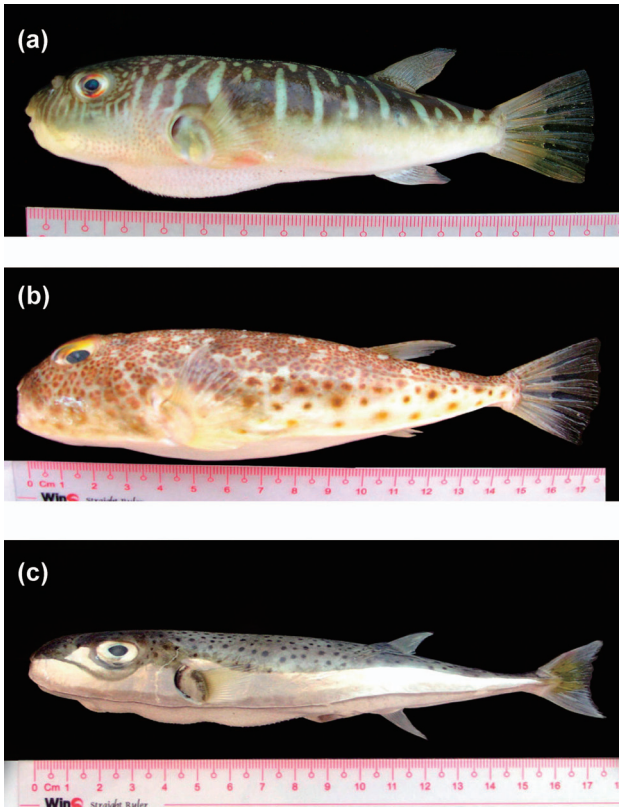

Fig. 1. Three species of puffers examined in the present study. (a) *Takifugu oblongus*, (b) *Torquigener gloerfelti*, (c) *Lagocephalus sceleratus*.

Results

Toxin component in puffers

Figure 3 shows an example of HPLC chromatograms obtained from muscle extract of *T. gloerfelti*. Both TTX and STX were detected in most of the specimens with the exceptions of *T. gloerfelti* specimens. 4*epi*TTX and anhydro TTX as well as neo-STX and dc-STX were also detected in almost all puffer extracts.

Figure 4 summarizes molecular ratio (%) of TTX toxins to total toxin (STXs and TTXs) in each organ of *T. gloerfelti* (n=30). TTX toxins accounted for about 95–98% of total toxins in all organs. The similar toxin proportion was recorded in the extracts of *T. oblongus* and *L. sceleratus* (data not shown). The present data in combination with a previous observation from a preliminary study on toxin in puffers from Vietnam (Dao unpubl.) indicate that TTX was a main component in puffers from Vietnam.

Individual variation of toxicity

As described above, main toxin component in Vietnamese puffer was found to be TTXs in the present study, and minor amount of toxicity due to PSP toxins of these specimens could be ignored. Table 2 shows average toxicity of the extracts from different organs of three puffer species, which was calculated from specific toxicity of each TTX toxin component and expressed in mouse unit (MU) (Nakamura and Yasumoto 1985).

In each organ, toxicity showed remarkable individual differences with very wide range in every species (Fig. 5). In *T. oblongus*, maximum toxicity was found in the liver (702.4 MU g^{-1}), while in *T. gloerfelti*, it was found in reproductive organ (977.9 MU g^{-1}). The extracts from muscle of three species were found to be toxic, though the level was different. Muscle of *L. sceleratus* was highly toxic (58.7±62.3 MU g^{-1}; range: 3.8–213 MU g^{-1}) and 83% of the specimens contained TTX over 10 MU g^{-1}. In contrast, the

Table 1. Puffer specimens collected in Khanh Hoa province in 2007.

Species	Date of sampling	Sampling location	No. of specimens	Body weight (g) Mean±SD	Body length (cm) Mean±SD
T. oblongus	Aug. 07	Van Ninh[1]	9	87.8±22.4	15.9±1.3
T. gloerfelti	Mar–May. 07	Cua Be[2]	30	177.5±98.7	19.3±3.3
	Aug–Sep. 07	Van Ninh[1]	30	175.9±52.7	19.1±1.8
L. sceleratus	Jul–Sep. 07	Van Ninh[1]	12	295.6±209.1	26.39±6.50

[1] Fish port in Van Ninh district, Khanh Hoa province (60 km north from Nha Trang city)
[2] Fish port in Nha Trang city

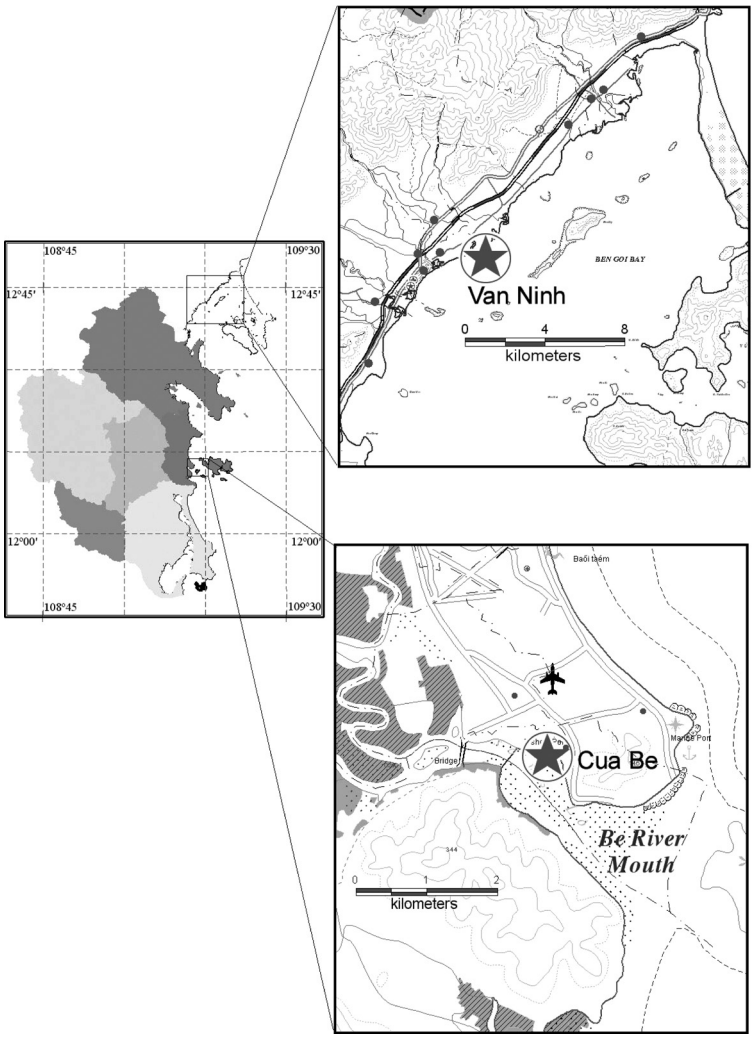

Fig. 2. Sampling places for puffers: Van Ninh and Cua Be Ports, Khanh Hoa Province.

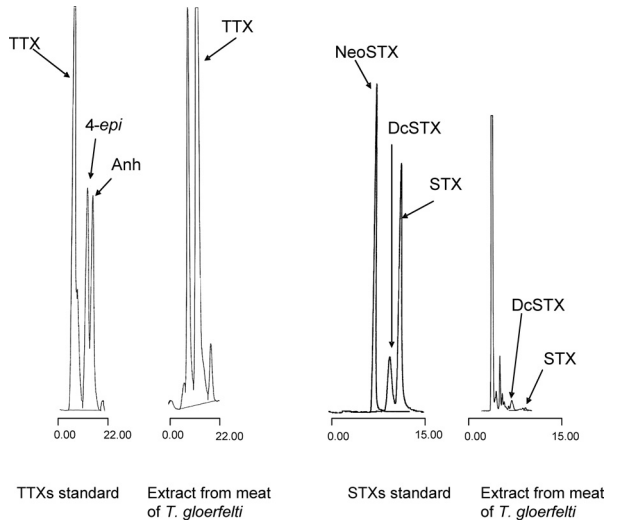

Fig. 3. HPLC chromatograms of the muscle extract of *T. gloerfelti*.

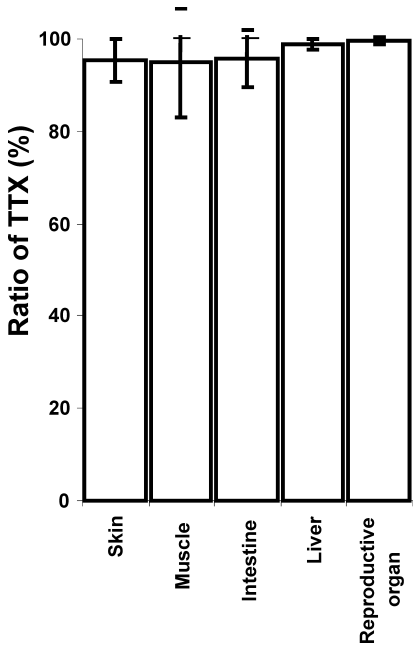

Fig. 4. Molecular ratio (%) of TTXs to total toxins (TTXs+STXs) in each organ of puffer *T. gloerfelti* (*n*=30).

Table 2. Mean, SD and range of toxicity (MU g^{-1}) in each body organ of puffer species.

Species	Sampling period	Skin		Meat		Intestine		Liver		Reproductive organ	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
T. oblongus	Aug. 07	22.4±12.7	0–40.1	6.4±7.3	0–22.1	98.4±93.0	0–221.8	178.1±239.3	0–702.4	54.8±106.2	0–333.9
T. gloerfelti	Mar–May. 07	14.0±14.0	0.5–53.9	2.6±3.5	0–14.7	11.1±13.2	0.3–54.5	154.4±200.8	6.1–782.3	123.2±240.6	0–977.9
	Aug. 07	12.6±13.9	0–52.3	2.5±3.6	0–14.6	9.0±11.2	0–36.5	80.8±103.8	3.6–387	62.3±109.5	0–458.7
L. sceleratus	Jul–Sep. 07	27.8±20.6	0.6–59.1	58.7±62.3	3.8–213	119.8±163	0.2–559.1	50.8±85.1	0–289.2	NT*	NT*

* No sample to test

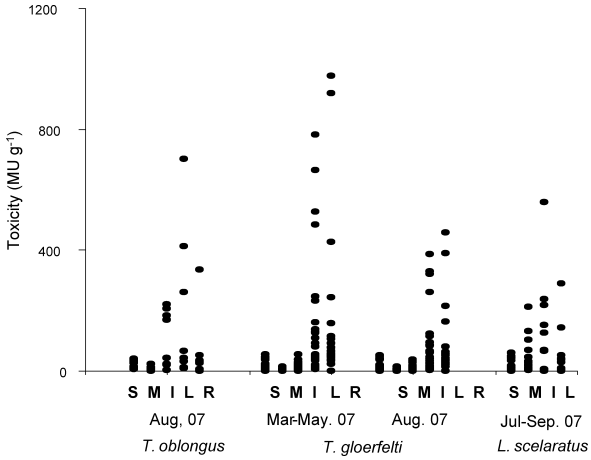

Fig. 5. Toxicity distribution in various organs of three puffer species. S, skin; M, muscle; I, intestine; L, liver; R, reproductive organ (ovary/testis).

muscle of *T. oblongus* and *T. gloerfelti* seems to be less toxic with lower frequency of toxic specimens (Table 3). The muscle often showed lowest toxicity, and the skin was the second lowest followed by the intestine, while the liver and reproductive organ always showed higher toxicity (Fig. 5). However, maximum toxicity in the muscle, especially the muscle of *L. sceleratus*, was far beyond the safety consumption level in Japan (Kodama and Sato 2005) (Table 2).

Among the skin, muscle and intestine of *T. gloerfelti*, no significant difference of toxicity was observed between two different sampling times in a year. In contrast, the liver and reproductive organ of several specimens of *T. gloerferti* collected in March–May 2007 showed much higher toxicity than those collected in August 2007. However, no significant difference was observed on the toxicity between the liver and reproductive organ in these two batch samples which showed toxicity higher than 10 MU g^{-1}.

Discussion

TTX was reported to be the main toxin component in *Takifugu* species collected in Japan (Kodama et al. 1984, Shiomi et al. 1985). The present study also showed that TTX was always the main toxin component in puffers collected from Vietnam. On the other hand, the present data was different from some other studies, which reported STX was major toxin component of some marine puffer species from the Philippines (Sato et al. 2000), freshwater puffers from Thailand (Kungsuwan et al. 1997, Sato et al. 1997), Bangladesh (Zaman et al. 1997). It is suggested that toxin component in puffers as well as other toxic organisms might have close relation with their environment, though the origin(s) of TTX as well as PSP toxins found in puffers had not been clear yet.

In June 2003, the Vietnamese government issued a strict

Table 3. Frequency (%) of puffer specimen containing toxicity beyond safety consumption level for TTX suggested in Japan (10 MU g^{-1}).

Species	Sampling time	*n*	Skin	Muscle	Intestine	Liver	Reproductive organ
T. oblongus	Aug. 07	9	78	22	89	89	56
T. gloerfelti	Mar–April. 07	30	50	3	37	93	70
	Aug. 07	30	40	3	30	87	70
L. sceleratus	Jul–Sep. 07	12	75	83	58	50	NT*

* No sample to test

ban for consumption of all puffer species. However, because of poor living standard, lack of scientific information and public awareness for the risk of toxins, uncontrolled local markets, and lack of organized monitoring system, fishermen communities processed puffers by themselves with several ways. Fishermen believed that it was safe to eat toxic puffer after specific processing such as removal of skin and viscera, salty-drying or fermenting to make fish sauce. In Japan, muscle and skin of some *Takifugu* and *Lagocephalus* species were accepted as edible, because these parts are almost non-toxic. In contrast, it had been reported that puffer fishes such as *L. lunaris* collected in South China Sea (Hashimoto 1979) and *Arothron* species collected in Philippines (Sato et al. 2000) often showed high toxicity in their muscle.

Specimens of three puffer species in this study showed a significant toxicity, comparable to some puffer species from other tropical countries such as the Philippines (Sato et al. 2000) and Thailand (Brillantes et al. 2003). The tendency of toxicity distribution among puffer body was also similar to that in puffers from Japan (Kodama et al. 1984) and India (Ghosh et al. 2004) although toxicity of Vietnamese puffers seems less toxic than Japanese puffers (Kodama et al. 1984). From public heath point of view, special attention should be paid to the fact that significant toxicity was found in consumed organs such as liver, reproductive organ (ovary) and muscle. Together with very wide individual variation of toxicity, these puffer species in Vietnam should be avoided to use as food resources. Especially, TTX in the muscle of *L. sceleratus* was frequently beyond the consumption level (10 MU g^{-1}), therefore, this species is dangerous for human consumption.

Ghosh et al. (2004) suggested that puffer ovaries and liver became highly toxic in mature period. As the number of the specimens was limited in this study, the toxicity between female and male was not clear. However, a large individual difference in toxicity suggested that the difference between sex seemed to be smaller than individual difference in the toxicity.

Public awareness and education about potential risk of puffers should be paid more attention, especially in local community.

Acknowledgements

We would like to thank to Prof. Dr. Keiichi Matsuura, National Museum of Nature and Science, Tokyo, Japan for correction of the scientific name of puffer. We also thank for the support from JSPS Multilateral Core University Program: Coastal Marine Science.

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