

Toxins of Pufferfish That Cause Human Intoxications

Osamu ARAKAWA¹, Deng-Fwu HWANG², Shigeto TANIYAMA¹
and Tomohiro TAKATANI¹

¹*Faculty of Fisheries, Nagasaki University,
1-14 Bunkyo-mach, Nagasaki 852-8521, Japan*

²*Department of Food Science, National Taiwan Ocean University,
Keelung, Taiwan, Republic of China*

Abstract—Many marine pufferfish possess a potent neurotoxin, tetrodotoxin (TTX). In general, they have strong toxicity in the liver and ovary, leading to a frequent occurrence of human poisonings. TTX is originally produced by marine bacteria and distributes over a wide variety of aquatic animals. In pufferfish, TTX is derived from the food chain that consists of these TTX-bearing organisms (i.e., their prey). The transfer, accumulation, and elimination mechanisms of TTX taken up into the pufferfish body via prey remain unclear. Recent studies have revealed that the liver of pufferfish has a specific TTX-uptake mechanism, and TTX introduced into the pufferfish body is first absorbed in the liver and then transferred to the skin through the circulatory system. This inter-tissue transfer and accumulation of TTX are greatly affected by the state of maturation. TTX-bearing organisms show extremely high resistance to TTX, and seem to possess TTX as a biological defense mechanism. Furthermore, TTX may be involved in the control of information transmission in the central nervous system of pufferfish.

TTX poisonings due to small scavenging gastropods have so far occurred in Taiwan and China. Recently, one such gastropod, *Nassarius glans*, caused food poisoning incidents in Kyushu, Japan. *N. glans* is highly toxic, and possesses a large amount of TTX not only in the viscera but also in the muscle. After 1990, a total of 9 poisoning incidents due to ingestion of boxfish (pufferfish of the family Ostraciidae) occurred in southwestern Japan, involving 13 patients and 1 death. The symptoms are very similar to those of parrotfish poisoning (a unique variety of food poisoning that has sporadically occurred in Japan), suggesting that the causative substance is a palytoxin (PTX)-like toxin as in the parrotfish poisoning. Freshwater pufferfish and some marine pufferfish possess paralytic shellfish poison (PSP) instead of or in addition to TTX, and may cause ‘paralytic shellfish poisoning by pufferfish’.

The toxins of the above mentioned fish and shellfish are all exogenous, and their toxicity may be greatly affected by a change in the marine environment, such as elevations in water temperature due to global warming. We need to enhance the information/collaboration network among East Asian countries and vigilantly monitor how our changing climate is affecting the toxicity and distributions of these organisms.

Keywords: tetrodotoxin, paralytic shellfish poison, palytoxin, pufferfish, gastropod, *Nassarius glans*, boxfish, *Ostracion immaculatus*

1. INTRODUCTION

Many Japanese know that pufferfish possess a fatal toxin. Nevertheless, they have a historical preference for eating pufferfish, and established unique food culture associated with this organism. However, as food poisonings due to ingestion of pufferfish were occurring very frequently, the Japanese Ministry of Health and Welfare (presently the Ministry of Health, Labour, and Welfare) published a guideline for edible pufferfish in 1983, with updates in 1993 and 1995 (Noguchi and Ebesu, 2001; Noguchi and Arakawa, 2008). Since then, accidents in specialist restaurants have been almost eliminated, but many cases of pufferfish poisoning continue to occur every year due to the consumption of home-made dishes with toxic portions, such as liver and ovary, which are prepared using wild fish that are caught recreationally. On the other hand, food poisonings due to small gastropods that possess the same toxin as pufferfish have occurred very frequently in Taiwan and China, where consumption of pufferfish is completely prohibited (Hwang and Noguchi, 2007; Hwang *et al.*, 2007). In the present paper, we review the property of 'pufferfish toxin' tetrodotoxin (TTX), species and toxic portions of TTX-bearing organisms, the accumulation mechanism and physiological function of TTX in pufferfish, and cases of human poisoning due to pufferfish. We also provide an introduction to TTX poisonings caused by marine organisms other than pufferfish, which are presently posing a food hygiene issue in Japan, and pufferfish poisonings due to toxins other than TTX.

2. PROPERTY OF TETRODOTOXIN (TTX)

Tetrodotoxin, a pufferfish toxin named after its order name Tetraodontiformes, is a potent neurotoxin of low molecular weight, whose unique structure (Fig. 1) was determined by three groups in 1964 (Tsuda *et al.*, 1964; Woodward, 1964; Goto *et al.*, 1965). Various TTX derivatives have so far been separated from pufferfish, newts, frogs, and other TTX-bearing organisms (Yotsu-Yamashita, 2001). High-purity TTX is insoluble not only in all sorts of organic solvents but also in water, though it becomes soluble in water when an acid is added. The toxin is stable in neutral to weakly acidic solutions and does not decompose by cooking (*i.e.*, the application of heat). TTX inhibits the conduction of action potential by selectively plugging sodium channels on the nerve/muscle membrane at extremely low concentrations (Narahashi, 2001). The lethal potency is 5000 to 6000 MU/mg [1 MU (mouse unit) is defined as the amount of toxin required to kill a 20-g male mouse within 30 min after intraperitoneal administration], and the minimum lethal dose (MLD) for humans is estimated to be approximately 10000 MU (≈ 2 mg) (Noguchi and Ebesu, 2001).

The main symptoms of human intoxication include numbness of lips, tongue and the limbs, paresthesia, dysarthria, respiratory distress; death can occur due to respiratory failure in most critical cases (Noguchi and Ebesu, 2001). When a poisoning occurs, it is essential to transport the patient immediately to a well-equipped hospital. At present, there is no antidote or specific medication for TTX, and no fundamental treatment besides facilitating elimination of the toxin from the body,

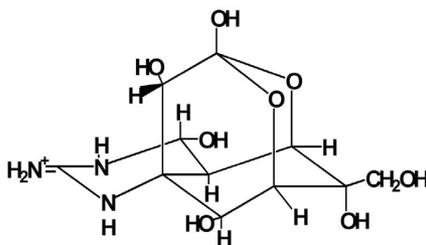


Fig. 1. Chemical structure of tetrodotoxin (TTX). Reprinted from *Marine Drugs* 6, Noguchi and Arakawa, Tetrodotoxin—distribution and accumulation in aquatic organisms, and cases of human intoxication, 220–242, 2008, Fig. 1.

and managing the respiratory/circulatory system properly using an artificial respirator. Although a monoclonal anti-TTX antibody has recently been developed (Kawatsu et al., 1997) and utilized as a chemical tool for research, it has little effect in clinical use.

3. DISTRIBUTION OF TTX IN AQUATIC ORGANISMS

Among the marine pufferfish inhabiting coastal waters of Japan, the following 22 species are listed as toxic; “kusafugu” *Takifugu niphobles*, “komonfugu” *T. poecilonotus*, “higanfugu” *T. pardalis*, “shosaifugu” *T. snyderi*, “mafugu” *T. porphyreus*, “karasu” *T. chinensis*, “mefugu” *T. obscurus*, “mushifugu” *T. exascurus*, “nameradamashi” *T. pseudommus*, “akamefugu” *T. chrysops*, “nashifugu” *T. vermicularis*, “torafugu” *T. rubripes*, “shimafugu” *T. xanthopterus*, “gomafugu” *T. stictonotus*, “shiroamifugu” *Tetraodon alboreticulatus*, “senninfugu” *Pleuranacanthus sceleratus*, “okinawafugu” *Chelonodon patoca*, “hoshifugu” *Arothron firmamentum*, “kitamakura” *Canthigaster rivulata*, “dokusabafugu” *Lagocephalus lunaris*, “kanafugu” *L. inermis*, “sansaifugu” *Takifugu flavidus* (Noguchi and Arakawa, 2008). All belong to the Tetraodontidae family, and pufferfish of Diodontidae and Ostraciidae possess no TTX at all. The toxic parts are different depending on species, which can be categorized as (1) muscle, testis and skin that are non-toxic (less than 10 MU/g) and edible; *T. rubripes*, *T. xanthopterus*, “shirosabafugu” *Logocephalus wheeleri*, etc., (2) skin is toxic, but muscle and testis are edible; *T. snyderi*, *T. porphyreus*, *T. vermicularis*, etc., (3) testis is also toxic, and only muscle is edible; *T. niphobles*, *T. poecilonotus*, *T. pardalis*, etc. In general, viscera, especially the liver and ovary are highly toxic (the toxicity often exceeds 1,000 MU/g), and the Japanese Ministry of Health, Labour, and Welfare has prohibited these organs from being used for food from all species of pufferfish.

The toxicity of Taiwanese marine pufferfish was extensively studied by one of the present authors (Hwang et al., 1992a). Among the 23 species examined, only two species, “kurosabafugu” *Logocephalus gloveri* and *L. wheeleri*, which are likely used as the ingredients for producing dried dressed fish fillets, were non-toxic in all tissues, whereas *L. lunaris*, “takifugu” *Takifugu oblongus*, and *T. nihpbles* that occasionally

cause food poisonings in Taiwan were highly toxic. *L. lunaris* from Thailand and Cambodia (Brillantes *et al.*, 2003; Ngy *et al.*, 2008a), and *T. oblongus* from Bangladesh and Cambodia (Mahmud *et al.*, 1999c; Ngy *et al.*, 2009) are also highly toxic, and considered as a potential causative species of pufferfish poisonings in these countries.

Small pufferfish inhabiting brackish water (Mahmud *et al.*, 1999a, b) or freshwater (Kungsuwan *et al.*, 1997; Sato *et al.*, 1997; Ngy *et al.*, 2008b) in Southeast Asia are also toxic. Toxicity of the skin is usually higher than that of the viscera in these pufferfish. The toxin of brackish water species was identified as TTX (Mahmud *et al.*, 1999a, b), but in the freshwater species, saxitoxins (STXs), toxins that belong to the paralytic shellfish poison (PSP) family (Deeds *et al.*, 2008), were detected as the main toxic principles (Kungsuwan *et al.*, 1997; Sato *et al.*, 1997; Ngy *et al.*, 2008b). In general, pufferfish shows large individual, regional, and seasonal variations in toxicity, and a fish of highly toxic species is not necessarily toxic. Thus the general public are often unaware of the danger, which has contributed to the frequent occurrence of pufferfish poisoning.

TTX was long believed to be present only in pufferfish. Since Mosher *et al.* (1965) identified a toxin from the eggs of the California newt *Taricha torosa* as TTX, however, TTX has been detected in a wide variety of animals, for example the goby *Yongeichthys criniger*, atelopid frogs, the blue-ringed octopus *Hapalochlaena maculosa*, the carnivorous gastropod *Charonia sauliae*, starfish of genus *Astropecten*, xanthid crabs, the horseshoe crab *Carcinoscorpius rotundicauda*, flatworms, and ribbon worms (Miyazawa and Noguchi, 2001; Hwang and Noguchi, 2007; Noguchi and Arakawa 2008). It is quite unlikely that these TTX-bearing organisms that belong to particular species in different phyla possess a common gene that codes for TTX production. Since the trumpet shell *C. sauliae* was found to accumulate TTX by ingesting toxic starfish (Noguchi *et al.*, 1982), the TTX of pufferfish has also been considered to be not endogenous, but to come from toxic food organisms via the food chain. In the 1980s, several studies were carried out to seek the primary origin of TTX in the food chain, and TTX productivity was found in certain species of marine bacteria including *Vibrio alginolyticus*, *Shewanella alga*, and *Alteromonas tetraodonis* that had been isolated from TTX-bearing organisms such as pufferfish, toxic starfish, the xanthid crab *Atergatis floridus*, and the red alga *Jania* sp. (Noguchi *et al.*, 1986; Yasumoto *et al.*, 1986; Narita *et al.*, 1987; Simidu *et al.*, 1987; Hashimoto *et al.*, 1990).

4. ACCUMULATION OF TTX IN PUFFERFISH

Many years of studies on TTX have revealed that (1) pufferfish toxicity shows remarkable individual and regional variations, (2) TTX is distributed over various organisms, including food animals of pufferfish, (3) the trumpet shell accumulates TTX by ingesting toxic starfish, (4) marine bacteria primarily produce TTX, (5) pufferfish become non-toxic when they are fed TTX-free diets in an environment in which the invasion of TTX-bearing organisms is completely prevented (Matsui *et al.*, 1982; Saito *et al.*, 1984; Noguchi *et al.*, 2004, 2006b) (Fig. 2), and (6) such non-toxic pufferfish efficiently accumulate TTX when orally administered TTX (Matsui *et al.*, 1981; Yamamori *et al.*, 2004; Honda *et al.*, 2005a; Noguchi *et al.*, 2006a; Kono

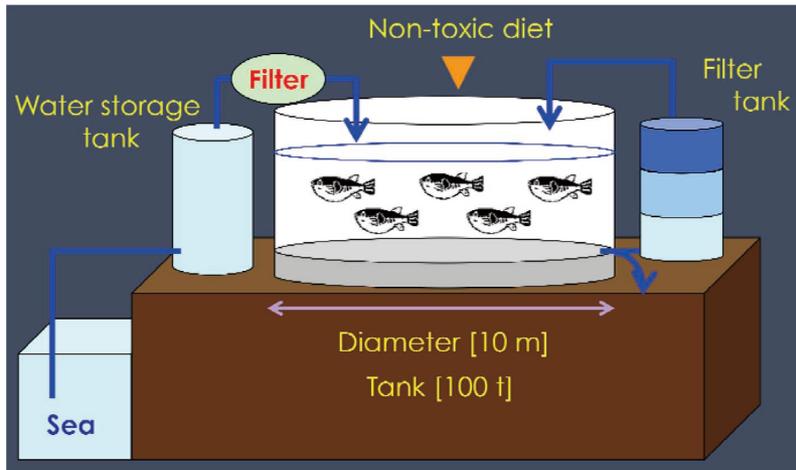


Fig. 2. Production of non-toxic pufferfish by land culture, in which seawater is thoroughly filtered before pouring into a tank to prevent toxic organisms from entering into it. Redrawn after *Japan Food Science*, 44, Arakawa et al., 42–47, 2005, Fig. 3. © JSFS.

et al., 2008), suggesting that pufferfish do not synthesize TTX, but accumulate it through the food chain, which starts from marine bacteria.

The transfer, accumulation, and elimination mechanisms of TTX taken up into the pufferfish body via food organisms remain unclear. Recently, we found that TTX administered intramuscularly to non-toxic cultured specimens of *T. rubripes*, which possesses TTX mainly in the liver in nature, was transferred first into the liver and then the skin via the blood (Ikeda et al., 2009). A similar transfer was observed when PSP was administered to non-toxic cultured specimens of the freshwater pufferfish *T. turgidus* that has PSP in the skin (Ngy et al., 2008b). The amount of toxin transferred to the liver, however, was very little in *T. turgidus*, and more than 90% of the toxin remaining in the body was transferred/accumulated in the skin. Interestingly, when *T. turgidus* specimens were administered the same dosage of TTX, all died within 3 to 4 h, and more than half of the TTX administered remained in the muscle in the dead specimens. Matsumoto/Nagashima et al. demonstrated that unlike general non-toxic fish, the liver tissue of *T. rubripes* is equipped with a specific TTX-uptake mechanism (Nagashima et al., 2003; Matsumoto et al., 2005, 2007), and using a pharmacokinetic model showed that TTX introduced into the pufferfish body is rapidly taken up into the liver via the blood (Matsumoto et al., 2008a, 2008b). These facts indicate that marine pufferfish that ingest TTX are endowed with a mechanism by which they transport TTX specifically and actively, and freshwater pufferfish that ingest PSP are endowed with a mechanism that processes PSP. TTX/PSP-binding proteins have been isolated from the blood plasma of marine pufferfish (Matsui et al., 2000; Yotsu-Yamashita et al., 2001), and may be involved in the transportation mechanism. Very recently, we investigated seasonal changes in tissue toxicity, as

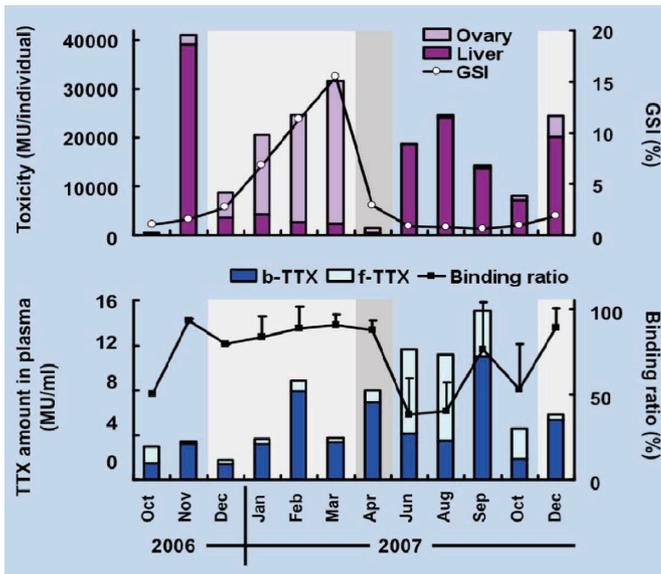


Fig. 3. Seasonal changes in the toxicity (MU/individual) of the skin (upper) and ovary/liver with GSI (middle), and in the TTX amount of blood plasma (lower) in the female specimens of *Takifugu poecilonotus*. The sum of free TTX (f-TTX) and TTX binding to high molecular-weight substances (b-TTX) was considered as a total TTX amount in plasma (p-TTX), and the percentage of b-TTX in p-TTX was calculated as the binding ratio. Data are shown by mean of each month (column or symbol on sequential line). Error bars (SD) for data other than the binding ratio are omitted to avoid confusion. Redrawn after *Toxicol.*, 55, Ikeda *et al.*, Maturation-associated change in toxicity of the pufferfish *Takifugu poecilonotus*, 289–297, 2010, Fig. 4, with permission from Elsevier.

well as the amount and forms of TTX in the blood plasma using wild specimens of the pufferfish *T. poecilonotus* from the Ariake Sea, and demonstrated that the maturation greatly affects the inter-tissue transfer and/or accumulation of TTX via the blood stream in nature (Ikeda *et al.*, 2010) (Fig. 3).

5. PHYSIOLOGIC FUNCTION OF TTX IN TTX-BEARING ORGANISMS

As described above, a wide variety of organisms including pufferfish possess TTX. Physiological functions of TTX in these organisms can be estimated by elucidating the distribution of TTX in their body. In marine pufferfish and flatworms, the amount of toxins in the eggs are generally very high (Miyazawa and Noguchi, 2001). In addition, pufferfish and newts have TTX-bearing glands or secretory cells (succiform cells) in their skin (Tanu *et al.*, 2002; Tsuruda *et al.*, 2002; Mahmud *et al.*, 2003a,b) (Fig. 4), and secrete TTX by external stimuli (Kodama *et al.*, 1985; Saito *et al.*, 1985a; Tsuruda *et al.*, 2002), suggesting that they possess TTX as a biologic defense agent to protect themselves or their eggs from predators. On the other hand, the blue-ringed octopus and ribbon worms possess TTX in the posterior salivary



Fig. 4. TTX-bearing gland observed in epidermis of the skin section in *Takifugu vermicularis* under light microscope ($\times 100$). The positive stain to TTX-antibody results in brown color. Stronger TTX antigen-antibody reaction was recognized at cytoplasm of the gland (arrow head). Modified from *Toxicon* **41**, Mahmud et al., Intra-tissue distribution of tetrodotoxin in two marine puffers *Takifugu vermicularis* and *Chelonodon patoca*, 13–18, 2003, Fig. 1, with permission from Elsevier.

gland and proboscis, respectively, and are believed to utilize the toxin to capture prey (Sheumack and Howden, 1978; Tanu et al., 2004). Recently, we observed that when non-toxic cultured pufferfish were fed with TTX-containing diets, their immune function was activated (Honda et al., 2005b), though the mechanism remains unclear.

TTX-bearing organisms such as toxic marine pufferfish, the goby *Y. criniger*, the xanthid crab *A. floridus*, and the newt *C. pyrrhogaster* show extremely high resistance to TTX, i.e., the MLD of TTX administered intraperitoneally to these animals is 300 to 1,000 times (more than 10,000 times in the newt) greater than that of mice (Noguchi and Hashimoto, 1973; Koyama et al., 1983; Saito et al., 1985b; Arakawa, 2001). In contrast, non-toxic marine pufferfish show medium resistance to TTX (MLD, 13 to 15 times greater than mice), and in general fish show resistance as low as mice (Saito et al., 1985b). The mechanism of TTX resistance in pufferfish and newts has been explained based mainly on the TTX-resistant sodium channels found in the animals, in which the aromatic amino acid commonly located in the p-loop region of domain I in TTX-sensitive sodium channels is replaced by a nonaromatic amino acid, resulting in their extremely low affinity to TTX (Kaneko et al., 1997; Yotsu-Yamashita et al., 2000; Venkatesh et al., 2005; Maruta et al., 2008). Garter snakes and clams can also acquire similar sodium channel mutation-based TTX/PSP resistance by interacting with their toxic food organisms, TTX-bearing newts and PSP-producing dinoflagellates, respectively (Geffeney et al., 2005; Bricejl et al., 2005).

Toxic small gastropods are also endowed with high resistance to TTX, and secrete TTX as defense or attack agent (Hwang et al., 1990a,b, 1992b). Interestingly, they were significantly attracted by TTX, while non-toxic species showed a negative response (Hwang et al., 2004). The more toxic species appeared to prefer TTX, indicating that TTX is an attractant for the toxic gastropods. Matsumura (1995) found that TTX was mostly distributed in the surface of pufferfish eggs, and might act as a

Table 1. Food poisonings due to animal natural toxins (Total score of 2002–2006, Ministry of Health, Labour and Welfare).

Causative food	Causative toxin	Number of incident	Number of patient	Number of death
Pufferfish of Tetraodontidae	Tetrodotoxin	166	223	13
Ciguateric fish	Ciguateric toxins	17	67	0
Gastropods of Buccinidae	Tetramine	16	38	0
Boxfish (Pufferfish of Ostraciidae)	Palytoxin-like toxin	3	6	0
Prickleback	Dinogunellin	1	4	0
Marine turtle	Unknown	1	1	0
Unknown		19	31	1
Total		223	370	14

Reproduced from Arakawa et al. *Koshu Eisei*, **73**(5), 323–326, 2009. Table 1. © IGAKU-SHOIN Ltd.

pheromone to attract the male fish.

Very recently, Sakakura et al. (unpublished) found that when TTX was administered to artificial-reared non-toxic juveniles of *T. rubripes*, their ecological behavior became similar to that of wild juveniles, and it was more difficult for predators to prey on them. This suggests a possibility that TTX is involved in the control of information transmission in the central nervous system of pufferfish. Sodium channel mutations and/or presence/absence of TTX in the cranial nervous system may exert a great influence on the physiology and ecology of pufferfish.

6. TTX INTOXICATION DUE TO PUFFERFISH

According to the statistics of Japanese Ministry of Health, Labour and Welfare (Table 1), 116 incidents of pufferfish poisoning has occurred in Japan, involving 223 patients and 13 deaths during the 5 years from 2002 to 2006. One of the more recent poisoning cases is described below. In October 2008, a 69 year-old male died at a hospital in Isahaya, Nagasaki Prefecture. He stated that he cooked a “usubahagi” (a sort of thread-sail filefish “kawahagi”), caught by himself, and ate its raw meat (sashimi) in a dip of the liver and soy sauce mixture. About 30 minutes after ingestion, he felt numbness in the limbs, and after 30 minutes, vomited, and fell into a coma, before being transported by an ambulance to the hospital. The doctor confirmed his death about 4 hours after ingestion, and initially provided the following diagnosis: ‘ciguatera due to the ingestion of “kawahagi” liver, a possibility of TTX is not denied’. Thereafter, it was determined that the patient cooked a “kinfugu” with the “usubahagi”, but the liver was missing among the leftovers. We investigated the leftovers, and revealed that the “usubahagi” was non-toxic, but the “kinfugu” was actually a highly toxic species, “komonfugu” *T. poecilonotus*, and 600 MU/g of TTX was detected in the skin. Furthermore, 0.7 MU/mL, 2 MU/mL, and 45 MU/g of TTX was detected in the blood, urine, and vomit of the patient, respectively, allowing us

to conclude that the present poisoning was a TTX intoxication due to the mistaken ingestion of *T. poecilonotus* liver.

To clarify the cause of pufferfish poisoning, identification of causative species, as well as investigation of leftover fish toxicity are essential. Although the species identification is usually carried out based on the morphological characteristics such as pattern of the skin, shape of the fins, and distribution of the small spines, several methods using proteins or genes have also been established (Chen and Hwang, 2002; Chen et al., 2002a,b, 2003, 2004; Ishizaki et al., 2005), and species can be identified even from a small tissue fragment. Analytical techniques of TTX have also been advanced, and TTX can be detected not only in the leftovers but also in the blood and urine of the patient (Kawatsu et al., 1999; Kurono et al., 2001; O'Leary et al., 2004; Akaki and Hatano, 2006; Tsai et al., 2006).

In Taiwan and China, although people do not eat pufferfish as often as the Japanese, many food poisoning cases due to ingestion of wild pufferfish have occurred. According to the records of TTX poisoning in Taiwan, that some cases are caused by the mistaken ingestion of the muscle tissue of a pufferfish species with toxic muscle, and by ingesting puffer roe that had been sold as fake dried mullet roe called "karasumi", or by ingesting a dried dressed fish fillet produced from toxic pufferfish by a food processing company (Du et al., 1999; Hwang et al., 2002a; Hsieh et al., 2002, 2003; Hwang and Noguchi, 2007). In countries outside of East Asia, people generally do not have a custom of eating pufferfish, and poisonings do not occur as frequently.

7. TTX INTOXICATION DUE TO MARINE ANIMALS OTHER THAN PUFFERFISH

In July 2007, a food poisoning incident due to the scavenging gastropod "kinshibai" *Nassarius glans* (Fig. 5) suddenly occurred in Nagasaki, Nagasaki Prefecture. The patient was a 60 year-old female, who developed symptoms such as a feverish feeling in the limbs, abdominal pain, and hectic flush and edema in the face 15 minutes after ingestion, and was administered an intravenous drop at a clinic nearby her home. Thereafter, her condition worsened, developing dyspnea, paralysis in the whole body, and mydriasis; she was finally transported to an emergency hospital. The patient required an artificial respirator for the first 3 days, but recovered enough to take breakfast on the 4th day. However, she unexpectedly relapsed after lunch, fell into respiratory arrest, and was equipped with the respirator again. Afterwards, she gradually recovered, and was discharged from the hospital 3 weeks later.

Immediately after the incident, we investigated the leftover gastropods, and detected a maximum of 4,290 MU/g of TTX in the cooked muscles and digestive glands of *N. glans*. Moreover, during the subsequent investigations, an extremely high concentration of TTX and a putative derivative of TTX, i.e., a maximum of 10,200 MU/g (15,100 MU/individual) in the viscera and 2,370 MU/g (9,860 MU/individual) in the muscle, was detected in *N. glans* specimens collected from the same sea area as the leftovers (Taniyama et al., 2009a) (Nagasaki specimens in Fig. 6). In the present intoxication, the symptoms once recovered recurred after taking meals.



Fig. 5. Scavenging gastropod “kinshibai” *Nassarius glans*. Reprinted from Arakawa *et al.*, *Koshu Eisei*, 73(5), 323–326, 2009. Fig. 2. © IGAKU-SHOIN Ltd.

Although the reason is not clear, it might be attributable to the situation where a highly toxic undigested tissue fragment of *N. glans* remaining in a digestive tract of the patient was digested and absorbed accompanying with the resumption of meals, and her respiratory center was again exposed to a high concentration of TTX. In July 2008, another poisoning incident due to *N. glans* occurred in Amakusa, Kumamoto Prefecture.

In China and Taiwan, people have a time-honored custom of eating small scavenging gastropods, and food poisoning due to these organisms has frequently occurred. At least 28 incidents were recorded during 1985–2004 in China, and 9 incidents during 1994–2006 in Taiwan, involving 233 patients and 24 deaths in total (Takatani *et al.*, 2005; Hwang *et al.*, 2007). In April 2004, a serious incident due to *N. glans* occurred in Taiwan, in which 2 of 6 poisoned patients died within 30 min after ingestion (Hwang *et al.*, 2005). The causative species in China was identified as *Zeuxis samiplicatus* (Sui *et al.*, 2002, 2003), while a total of 14 species of Nassariidae, Naticidae, and Olividae including *N. glans* were reported as the responsible gastropods for the poisonings in Taiwan (Hwang *et al.*, 1995, 2002b, 2003, 2005, 2007; Shiu *et al.*, 2003). Among them, *N. glans*, *N. papillosus*, *Z. scalaris*, and *Oliva miniacea* are also distributed in Japanese coastal waters.

From 1979 to 1987, 3 incidents of TTX intoxication due to the carnivorous gastropod *C. sauliae* occurred in Shizuoka, Wakayama and Miyazaki Prefectures, respectively (Narita *et al.*, 1981; Maruyama *et al.*, 1983). In this species, the toxin is localized at the digestive gland, and a total of 4 persons who had eaten not only the muscles but also gland were poisoned. In Taiwan, where at least 4 species of TTX-bearing gobies, *Yongeichthys nebulosus*, *Sillago japonica*, *Prachaeturichtys palynena*, and *Radigobius caninus*, are found, several fatal poisoning incidents and frequent deaths of duck, both due to ingesting the gobies have occurred (Lin *et al.*, 1996, 2000). The highest toxicity scores of poisoning-related specimens of *Y. nebulosus* and *S. japonica* were 7,650 and 1,460 MU per individual, respectively (Lin *et al.*, 1999). In some Southeast Asian countries, eggs of the horseshoe crab are used as a food, which can occasionally cause food poisonings. TTX and/or PSP were detected in the

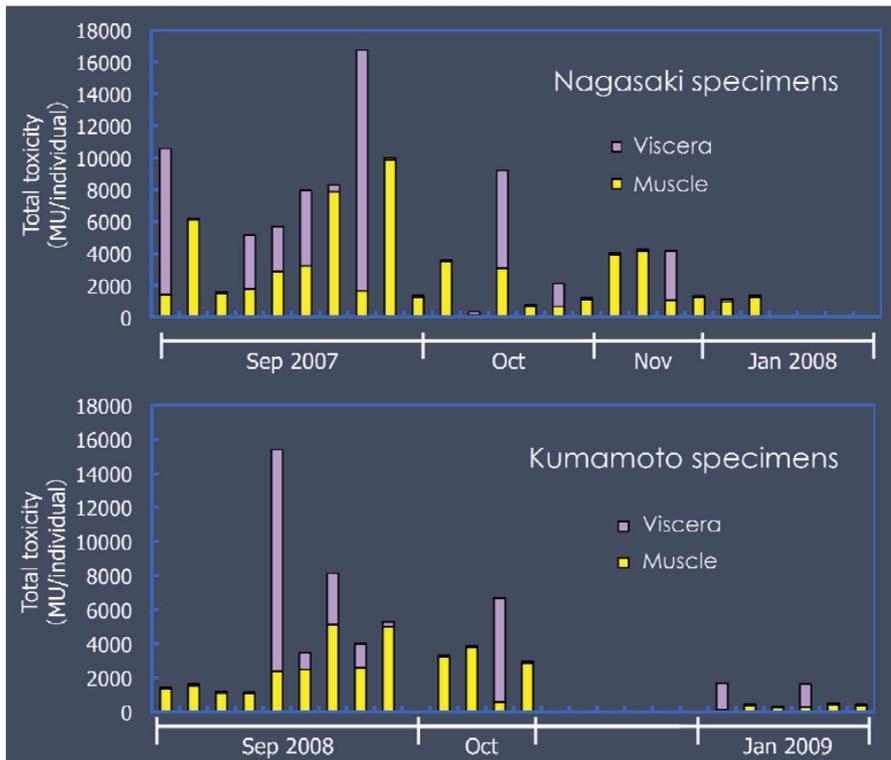


Fig. 6. Toxicity of *N. glans* specimens collected from Tachibana Bay, Nagasaki Prefecture (upper) and Miyanakawachi Bay, Kumamoto Prefecture (lower).

eggs and hepatic caecum of *Carcinoscorpius rotundicauda*, indicating that either of them or both are the causative agents (Fusetani et al., 1983; Kungsuwan et al., 1987; Tanu and Noguchi, 1999; Ngy et al., 2007).

8. PUFFERFISH POISONING DUE TO DIFFERENT TOXINS FROM TTX

In the Goto Islands, Nagasaki Prefecture, broiled boxfish with miso has long been eaten as a local delicacy. From October 1990 to October 2008, however, a total of 9 poisoning incidents due to ingestion of the dish occurred in Kagoshima, Nagasaki, Mie, and Miyazaki Prefectures, involving 13 patients and 1 death (Taniyama et al., 2009b). The causative species was identified as “hakofugu” *Ostracion immaculatus* (Fig. 7) from morphologic observations of the leftover fish in 2 of the incidents. *O. immaculatus* belongs to Tetraodontiformes, and is treated as pufferfish from a food hygienic point of view in Japan. A similar food poisoning due to boxfish has also occurred in Taiwan (Chen et al., 2001).

A description of the fatal boxfish poisoning that occurred in Japan are as follows.

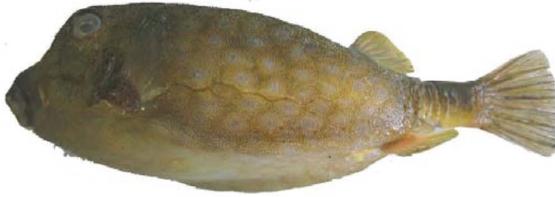


Fig. 7. Boxfish “hakofugu” *Ostracion immaculatus*. Reprinted from Arakawa *et al.* *Koshu Eisei*, **73**(5), 323–326, 2009. Fig. 3. © IGA KU-SHOIN Ltd.

In August 2007, 4 individuals consumed broiled boxfish with miso in Goto, Nagasaki Prefecture, and 2 of them were poisoned. One patient exhibited lower-back pain and myoglobinuria, however the symptoms were relatively mild, and he recovered within 2–3 days. The other patient, however, developed severe muscle pain due to rhabdomyolysis, and fell into dysstasia. Moreover, he exhibited respiratory distress and myoglobinuria, and then died about 2 weeks later. Although the boxfish “umisuzume” *Lactoria diaphana* was suspected as a causative species, it could not be identified, because all of the leftovers had been disposed.

Boxfish poisoning is very similar to parrotfish poisoning (a unique variety of food poisoning that has sporadically occurred in Japan) (Noguchi *et al.*, 1987; Arakawa *et al.*, 1992; Taniyama *et al.*, 2003a), and patients commonly show symptoms such as severe muscle pain accompanied with myoglobinuria and an abnormal rise of serum creatine phosphokinase (CPK), and the time course from the onset of the symptoms to recovery or death is relatively long. The causative substance is believed to be a palytoxin (PTX)-like toxin, as in the parrotfish poisoning (Noguchi *et al.*, 1987; Taniyama *et al.*, 2009b). A tropical/subtropical dinoflagellate *Ostreopsis* sp. (Fukuyo, 1981) is presumed to be one of the origins of the toxin, which has recently been found to inhabit the coastal waters of Kyushu and Honshu Islands in Japan (Taniyama *et al.*, 2003b; Sagara, 2008). Bangladeshi freshwater pufferfish also possess PTX-like toxin, and have frequently caused food poisonings in the country (Mahmud *et al.*, 2000; Taniyama *et al.*, 2001).

Small pufferfish inhabiting rivers or inland waters in Southeast Asia such as *Tetraodon fangi*, *T. leirus*, *T. suwatii*, and *T. turgidus* possess PSP mainly in their skin (Kungsuwan *et al.*, 1997; Sato *et al.*, 1997; Zaman *et al.*, 1997; Ngy *et al.*, 2008b). They are imported to Japan, and sold for ornamental purposes, but not used for food. In Thailand and Cambodia, however, food poisoning incidents due to the freshwater pufferfish have occasionally occurred with some fatalities. PSP is a group of neurotoxins produced by certain species of dinoflagellates, and the main component, STX, has almost equivalent molecular size and action mechanism to TTX (Deeds *et al.*, 2008). Therefore, the symptoms of freshwater pufferfish poisoning is very similar to those of marine pufferfish poisoning, i.e., TTX poisoning. Floridian *Spherooides* pufferfish possess a large amount of PSP in the muscle, and caused 28 poisoning cases

during 2002–2004 (Landsberg et al., 2006). Several marine pufferfish from the Philippines (Sato et al., 2000) and “hoshifugu” *Arothron firmamentum* from Japanese coastal waters (Nakashima et al., 2004) are also known to possess PSP as a main toxin component in addition to TTX.

9. CONCLUSION

‘Pufferfish toxin’ generally indicates TTX, but as described above, gastropod poisoning due to TTX, or shellfish toxin poisoning and parrotfish poisoning-like poisoning due to pufferfish frequently occur, posing a great food hygienic issue in East and Southeast Asian countries. These toxins are all exogenous, and both pufferfish and gastropods are considered to obtain them from their toxic prey organisms, and accumulate the toxin in specific organs. Therefore, the toxicity of these toxic fish and shellfish may be greatly affected by a change in the marine environment, such as elevations in water temperature due to global warming. We need to enhance the information/collaboration network among East Asian countries to keep our eye on the diversity of TTX-bearing organisms, or of the toxins that pufferfish possess.

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O. Arakawa (e-mail: arakawa@nagasaki-u.ac.jp), D.-F. Hwang, S. Taniyama and T. Takatani