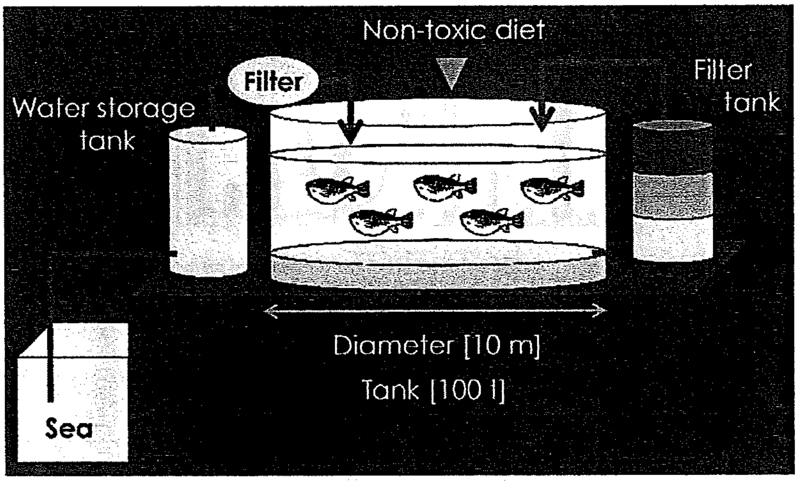

Fig. 2. Production of non-toxic pufferfish by land culture, in which seawater is thoroughly filtered before pouring into a tank to prevent toxic organisms from entering into it. Redrawn after *Japan Food Science*, **44**, Arakawa et al., 42–47, 2005, Fig. 3. © JSFS.

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

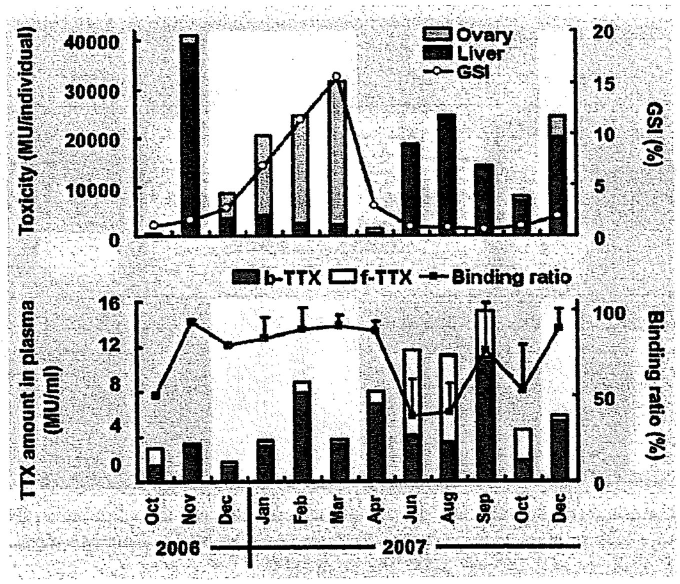

Fig. 3. Seasonal changes in the toxicity (MU/individual) of the skin (upper) and ovary/liver with GSI (middle), and in the TTX amount of blood plasma (lower) in the female specimens of *Takifugu poecilonotus*. The sum of free TTX (f-TTX) and TTX binding to high molecular-weight substances (b-TTX) was 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 *Toxicon*, 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

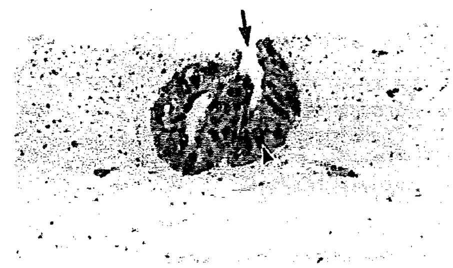

Fig. 4. TTX-bearing gland observed in epidermis of the skin section in *Takifugu vermicularis* under light microscope (×100). The positive stain to TTX-antibody results in brown color. Stronger TTX antigen-antibody reaction was recognized at cytoplasm of the gland (arrow head). Modified from *Toxicon* **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.

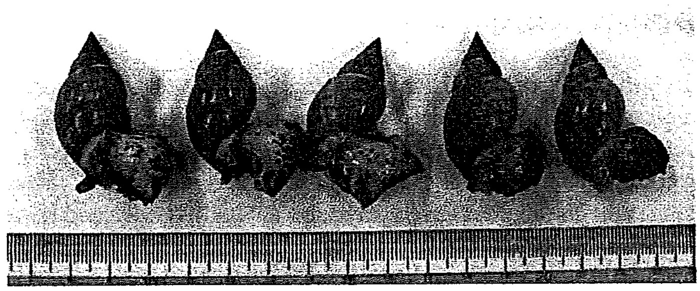

Fig. 5. Scavenging gastropod "kinshibai" *Nassarius glans*. Reprinted from Arakawa et al., *Koshu Eisei*, 73(5), 323–326, 2009. Fig. 2. © IGAKU-SHOIN Ltd.

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 samiplicutus* (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 Prifectures, 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

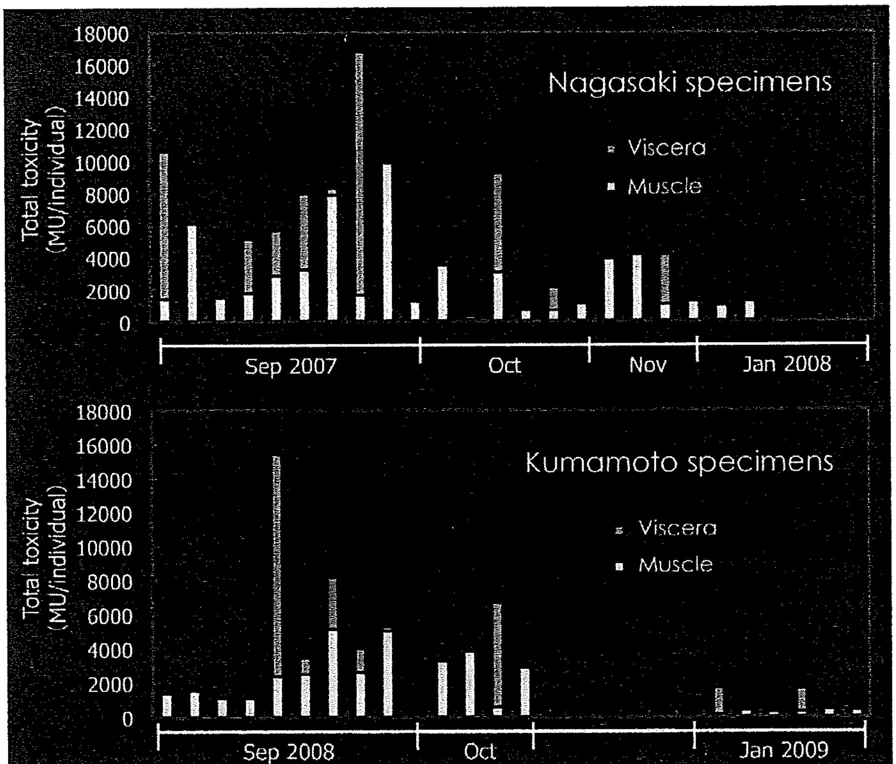

Fig. 6. Toxicity of *N. glans* specimens collected from Tachibana Bay, Nagasaki Prefecture (upper) and Miyanokawachi Bay, Kumamoto Prefecture (lower).

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.

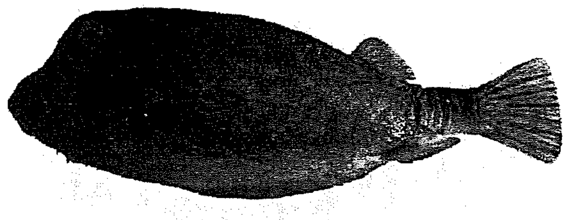

Fig. 7. Boxfish "hakofugu" *Ostracion immaculatus*. Reprinted from Arakawa et al. *Koshu Eisei*, 73(5), 323–326, 2009. Fig. 3. © IGAKU-SHOIN Ltd.

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. leiurus*, *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 *Sphoeroides* 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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特 集 食中毒の変遷と現状

9. フグ毒による食中毒

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フグ科の多くのフグは，強力な神経毒であるフグ毒テトロドトキシン（TTX）を保有する。通常，海産フグは肝臓と卵巣，汽水フグや淡水フグは皮の毒性が高い。1964 年以降，フグのほか極めて多様な生物から TTX が検出され，フグは海洋細菌を始点とする食物連鎖を介して TTX を摂取・蓄積することが明らかとなってきた。日本や中国，台湾では，フグあるいは小型巻貝によるヒトの TTX 中毒が頻発しており，多くの死者を出している。一方，フグは麻痺性貝毒（PSP）など TTX とは異なる毒を持つ場合があり，これによる中毒も発生している。

Key Words：フグ／小型巻貝／テトロドトキシン（TTX）／麻痺性貝毒（PSP）／食中毒

I はじめに

動物性自然毒起源の食中毒の中で，日本ではフグ毒テトロドトキシン(TTX)（図1）によるものが最も多い[1]。TTX は特異な化学構造を持つ低分子の神経毒で，これまでに種々の誘導体が，フグやイモリ，カエルから分離されている[2]。神経膜のナトリウムチャンネルを特異的に塞ぐことにより活動電位の伝導を阻害する[3]。毒力は 5,000～6,000MU/mg〔1 MU（マウスユニット）とは，腹腔内投与により体重 20g のマウスを 30 分で死亡させる毒量〕で，ヒトに対する最少致死量(MLD) は 10,000 MU（約 2 mg）と推定されている[1,4]。ヒトの主な中毒症状は，唇，舌の先，四肢のしびれや知覚麻痺，言語障害，呼吸困難などで重篤な場合は呼吸麻痺で死亡する[1,4]。

日本人は昔からフグを好んで食べ，独自のフグ食文化を築き上げてきたが，現在でもこれによる食中毒があとを絶たない。中毒を避け，フグ食の安全・安心を確保するためには，フグ毒あるいはそれを保有する生物に関する根本的な理解が不可欠である。本稿では，水生生物における TTX の分布，蓄積機構，生理機能について解説するとともに，TTX によるヒトの中毒事例，すなわち日本で多発するフグ中毒や，中国や台湾で頻発している小型巻貝による中毒，さらに TTX とは異なる毒を持つフグによる中毒について紹介する。

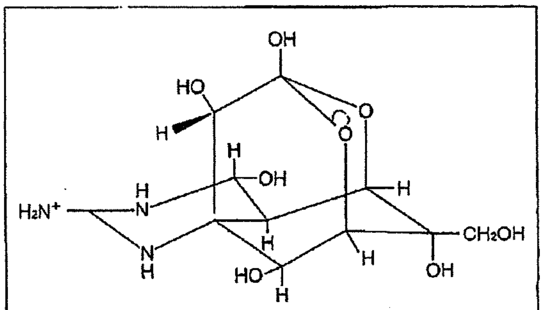

図1 TTX の化学構造

TTX は低分子（分子量 319）の神経毒で，その特異な化学構造は 1964 年に結晶の X 線解析により決定された。

Food poisonings due to pufferfish toxin
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Ⅱ　水生生物におけるTTXの分布

1．フグにおけるTTXの分布

日本近海産フグの毒性については，1940年代に広範な調査が行われ，21種中14種が有毒とされた。その後さらに8種のフグが有毒種に加えられ，現在，計22種がTTXを保有するフグとしてリストアップされている（表1）[5]。それらはいずれもフグ科に属しており，ハリセンボン科およびハコフグ科のフグは全くTTXを持たない。東南アジア産の汽水フグや淡水フグも有毒であることが知られている。汽水種の毒はTTXであるが，淡水種はTTXの代わりに麻痺性貝毒（PSP）[1]を保有する（この点については第Ⅴ章で詳しく述べる）。通常，フグの毒性には極めて大きな個体差や地域差，季節変動がみられ，有毒種とされるフグでも常に高毒性とは限らない。このことがかえって油断を招き，フグ中毒が多発する一因ともなっている。

フグ体内におけるTTX（またはPSP）の分布は種によって異なる（表1）。海産フグでは，通常肝臓と卵巣が最も高い毒性を示し，腸と皮がそれに次ぐ。筋肉と精巣はドクサバフグなど1部の例外を除き無毒または弱毒で，有毒種であっても多くのフグで食用が認められている。これに対し沖縄や奄美の沿岸域ないし汽水域に生息するオキナワフグや東南アジア産の汽水フグおよび淡水フグは，皮が高毒性を示す。近年，抗TTX抗体を用いる免疫組織化学的研究により，ナシフグ，オキナワフグなど皮が有毒なフグは，同部位にTTXを保有する腺組織もしくは分泌細胞を持つことが明らかとなっている[5]。

2．フグ以外の生物におけるTTXの分布

TTXはフグのみが持つ毒と考えられていたが，1964年にカリフォルニアイモリの毒がTTXと同定されて以来，ツムギハゼ，*Atelopus* 属のカエル，ヒョウモンダコ，肉食性巻貝ボウシュウボラ，*Astropecten* 属のヒトデ，スベスベマンジュウガニ，ヒラムシ，ヒモムシなど，多様な生物にTTXが見出されてきた[5,6]。これらの分類学的に類縁関係のない生物の特定の種のみがTTX産生遺伝子を共有しているとは考えにくい。ボウシュウボラについては，有毒ヒトデを食べることによりTTXを蓄積することが明らかにされており，後述のようにフグの毒化も内因性ではなく，食物連鎖を介する外因性のものと考えられるようになった。食物連鎖の出発点，すなわちTTXの起源生物については1980年代に検討がなされ，フグ，有毒ヒトデ，スベスベマンジュウガニ，紅藻ヒメモサヅキなどから分離された数種の海洋細菌（*Vibrio alginolyticus*, *Shewanella alga*, *Alteromonas tetraodonis* など）に相次いでTTX産生能が見出された[5,6]。

Ⅲ　フグにおけるTTXの蓄積と生理機能

1．有毒餌生物からのTTXの取り込み

前述にように，①フグの毒性には著しい個体差や地域差がみられること ②TTXはフグ以外の生物（フグの餌生物）にも広く分布すること ③ボウシュウボラについては，有毒ヒトデを食べることによりTTXを蓄積すること ④元来，海洋細菌がTTXを産生していることなどが順次明らかとなり，「フグの毒化は細菌起源の食物連鎖を介する外因性のものである」との説が有力となった。この説が正しければ，フグは有毒餌生物を遮断して無毒の餌で飼育すれば無毒になると考えられる。筆者らはこの点を明らかにするため，日本各地から‘囲い養殖法’（フグを網生け簀または陸上水槽に囲い込むことによって有毒餌生物から隔離する方法）（図2）で養殖したトラフグを集め，マウスを用いる生物試験法により，主に肝臓の毒性を調査した。その数は計5,000個体以上に及ぶものであるが，1個体も毒性は検出されなかった[7]。さらに1部の個体については液体クロマトグラフィー質量分析法（LC/MS）により微量の毒の有無についても検討したが，1個体もTTXは検出されな

TTX（テトロドトキシン）
PSP（麻痺性貝毒）
MLD（最少致死量）
LC/MS（液体クロマトグラフィー質量分析法）

表1　TTXを対象としたフグ類の毒性

科	生息域	種	最高毒力*					
			卵巣	精巣	肝臓	皮	腸	筋肉
フグ科	日本近海	クサフグ	●	○	●	◎	●	○
		コモンフグ	●	◎	●	◎	◎	○
		ヒガンフグ	●	○	●	◎	◎	×
		ショウサイフグ	●	×	●	◎	◎	○
		マフグ	●	×	●	◎	◎	×
		カラス	●	−	●	−	−	−
		メフグ	●	×	◎	◎	◎	×
		ムシフグ	●	×	◎	◎	−	×
		ナメラダマシ	●	×	○	○	○	×
		アカメフグ	◎	×	◎	◎	○	×
		ナシフグ	◎	×	◎	◎	○	×
		トラフグ	◎	×	◎	×	○	×
		シマフグ	◎	×	◎	×	○	×
		ゴマフグ	◎	×	◎	○	×	×
		シロアミフグ	●	−	○	○	◎	○
		センニンフグ	●	−	○	○	◎	○
		オキナワフグ**	◎	◎	◎	●	−	◎
		ホシフグ***	◎	×	×	○	×	×
		キタマクラ	×	−	○	◎	○	×
		ドクサバフグ	×	×	×	◎	×	●
		カナフグ	×	×	◎	×	×	×
		シロサバフグ	×	×	×	×	×	×
		クロサバフグ	×	×	×	×	×	×
		ヨリトフグ	×	×	×	×	×	×
	中国近海	サンサイフグ	●	◎	●	◎	◎	○
	タイ汽水域	ミドリフグ	−	−	×	◎	○	○
		ハチノジフグ	−	−	×	◎	×	×
ハリセンボン科	日本近海	ハリセンボン	×	−	×	×	×	×
		イシガキフグ	×	−	×	×	×	×
ハコフグ科	日本近海	ハコフグ	×	×	×	×	×	×
		ウミスズメ	×	×	×	×	×	×
		イトマキフグ	×	×	×	×	×	×

*×：10 MU/g未満（無毒）；○：10～100 MU/g（弱毒）；◎：100～1,000 MU/g（強毒）；●：1,000 MU/g以上（猛毒）；−：測定データなし

**汽水域にも生息

***皮の毒はTTXであるが，卵巣の毒の主成分はPSP

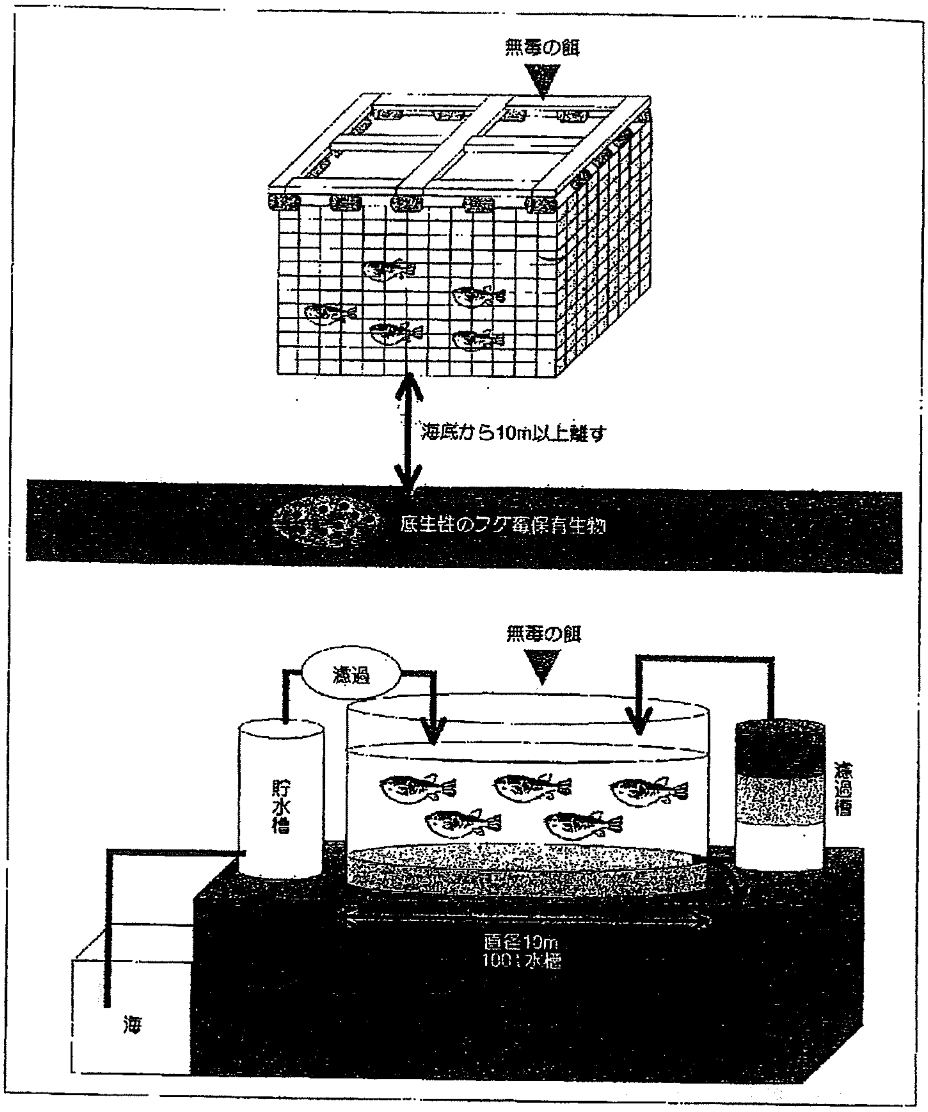

図2　トラフグの囲い養殖法

底生性の有毒餌生物を遮断した上で無毒の餌を与えて飼育する養殖法を‘囲い養殖法’と称している。これにより無毒のフグを生産することが可能である。網生け簀養殖(上)と陸上養殖(下)の2つの形態がある。

かった。

一方，毒を持たない一般の魚は致死量未満のTTXを餌に混ぜて投与しても，すぐに排泄もしくは分解して蓄積することはないが，無毒の養殖トラフグや養殖クサフグに，同様にTTXを与えると，肝臓や卵巣に効率良くそれを蓄積する[5)6)]。他方，養殖トラフグにPSPを投与すると，蓄積はするがその量はTTXの場合の2～5割程度と少ない。TTXを持つ海産フグはTTXを選択的に蓄積する能力があるといえる。

以上の知見から，有毒フグのTTXが内因性ではなく餌由来であることは明らかである。フグに共生もしくは寄生するTTX産生細菌がフグ体内でTTXを産生し，フグがそれを直接取り込んで毒化

するというルートも考えられるが，食物連鎖による生物濃縮を経ないとすると，細菌の産生するTTX量は天然フグの極めて高い毒量を説明するには少なすぎるように思われる。さらに無菌でない囲い養殖フグ5,000個体以上が無毒であったことを考慮すれば，フグ腸内で細菌が産生したTTXのフグのTTX蓄積に対する寄与は，あったとしてもごくわずかで無視しうるものであろう。

2．各組織へのTTXの移行・蓄積

フグの毒化は細菌から始まる食物連鎖で説明することが可能となった。しかしながら，有毒餌生物を介してフグ体内に取り込まれたあとのTTXの存在形態や動態に関しては，いまだに不明な部分が少なくない。最近筆者らは，天然では主に肝臓にTTXを保有するトラフグの無毒養殖個体の筋肉にTTXを注射投与すると，血液を介して速やかにTTXが皮や肝臓に移行することを見出した。PSPを皮に保有する淡水フグ(*Tetraodon turgidus*)の無毒養殖個体にPSPを投与した場合も同様の移行が観察されるが，この場合，肝臓への移行量はわずかで，最終的に体内に残存した毒の9割以上が皮に移行・蓄積した[8]。一方，海産フグ肝臓の培養組織が *in vitro* で著量のTTXを取り込むとの報告もある[9]。無毒の一般魚ではこのような現象はみられず，明らかにフグの肝臓や皮には特異なTTX/PSP蓄積機構が存在する。関連して，海産フグの血漿中にはTTX結合性タンパク質の存在が見出されており[10]，このような高分子物質がTTXの輸送や蓄積に深く関わっているものと推察される。

3．TTXの生理機能

前述のように，フグをはじめ非常に多様な生物がTTXを保有する。これらの生物においてTTXの体内分布を知ることにより，TTXの機能を推定することが可能である。海産フグやヒラムシは卵の毒量が非常に高い。またフグやイモリは，皮に腺組織または分泌細胞を持ち，外的刺激によりTTXを分泌することから，彼らは卵や自身を外敵の捕食から守る防御物質としてTTXを保有している可能性がある[6][11]。一方，ヒョウモンダコは後

表2　TTX保有生物と非保有生物のTTXに対する抵抗性

生物	種	MLD (MU/20g)*
フグ以外のTTX保有生物		
魚類	ツムギハゼ	300以上
両生類	ニホンイモリ	10,000以上
甲殻類	スベスベマンジュウガニ	1,000
フグ類		
フグ科・有毒	クサフグ	700～750
	ヒガンフグ	500～550
	トラフグ（養殖）	300～500
フグ科・通常無毒	シロサバフグ	15～18
	クロサバフグ	19～20
	ヨリトフグ	13～15
ハコフグ科・無毒	ハコフグ	0.9～1.3
一般魚類	イシガキダイ	0.8～0.9
	イシダイ	0.8～1.8
	メジナ	0.3～0.5
ほ乳類	マウス	1

*腹腔内投与におけるTTXの最少致死量（MLD；MU/20g体重）

部唾液腺に，ヒモムシは口吻にTTXをもち，餌生物の捕獲にそれを利用していると考えられる[6]。関連して，最近筆者らはその機構は不明であるが，養殖フグにフグ毒添加飼料を与えて飼育すると免疫機能が活性化することを見出している[12]。

TTXを保有する有毒海産フグ，ツムギハゼ，スベスベマンジュウガニ，およびニホンイモリはTTXに対して極めて高い抵抗性を示す[5][6]。すなわちこれらの生物に腹腔内投与した場合のTTXのMLDは，マウスの300～1,000倍(イモリでは10,000倍以上)に達する(表2)。これに対し，無毒の海産フグは中程度(MLDがマウスの13～15倍)，一般魚はマウスと同程度の低い抵抗性を示す。有毒フグやイモリのTTX抵抗性発現メカニズムのひとつとして，彼らがTTX耐性型のナトリウムチャンネルを保有していることが上げられる[13]。TTX保有イモリを補食するある種のヘビやPSPで毒化する二枚貝は，TTXまたはPSPに曝されることでナトリウムチャンネルをコードする遺伝子に変異を起こし，TTX/PSP抵抗性を獲得するとの報告もある[14][15]。

ごく最近筆者の共同研究者らは，無毒トラフグ人工種苗にTTXを投与すると行動生態が天然魚に近くなり，捕食魚による食害を受けにくくなることを見出した。このことはフグの中枢神経系においてTTXが情報伝達の制御に関わっている可能性を示唆している。脳神経系におけるナトリウムチャンネルの変異やTTXの有無がフグの生理・生態に大きな影響を及ぼしている可能性がある。

Ⅳ　TTXによるヒトの中毒事例

1．フグによる中毒

我々日本人の多くは，フグ，特にその肝(きも)には致死的な毒があることを知っている。にもかかわらず昔から，'秘伝の毒抜き'を施すなどして多くの食通があえて肝を食してきた。しかしながらこれによる食中毒もあとを絶たず，厚生省(現厚生労働省)は1983年12月に「フグの衛生確保について」の通知を出し，あらゆるフグの肝を食用に供することを禁止した(現時点では無毒養殖フグの肝も食用不可)。この通知以降，専門店での事故はほとんどなくなったが，素人が自分で釣ったフグや知人から譲り受けたフグを自ら調理し，肝まで食べて中毒する事例がいまだにあとを絶たない。厚生労働省の統計によれば，2002～2006年の5年間において，フグによるTTX中毒の患者数は223名で，うち13名が死亡している(表3)。中毒の一例[4]を記すと次のようである。1996

表3　動物性自然毒による食中毒発生状況
(厚生労働省統計 2002～2006年の合計値)

原因食品	原因毒	事件数	患者数	死者数
フグ(フグ科)	テトロドトキシン	166	223	13
シガテラ毒魚(バラフエダイ，バラハタ等)	シガテラ毒	17	67	0
エゾバイ科巻貝(エゾボラモドキ，ヒメエゾボラ等)	テトラミン	16	38	0
ハコフグ	パリトキシン様毒	3	6	0
ナガヅカ	ジノグネリン	1	4	0
ウミガメ	不明	1	1	0
不明		19	31	1
計		223	370	14