FISHERIES SCIENCE 2000; **66**: 547–552

Original Article

Toxicity of Taiwanese gobies

SHIN-JUNG LIN,[1] DENG-FWU HWANG,[1,*] KWANG-TSAO SHAO[2] AND SEN-SHYONG JENG[1]

[1]*Department of Food Science, National Taiwan Ocean University, Keelung and* [2]*Institute of Zoology, Academia Sinica, Taipei, Taiwan, ROC*

SUMMARY: Recently, three paralytic food poisoning incidents due to ingestion of the goby *Yongeichthys nebulosus* occurred in Taiwan. Therefore, intensive investigation of toxic goby was performed from August 1996 to July 1998. More than 300 specimens of 12 species of gobies were collected from 15 locations and examined for toxicity. The specimens of three species of gobies, *Y. nebulosus, Prachaeturichtys palynema,* and *Radigobius caninus,* collected from Pingtung, Penghu, Kaohsiung, Miaoli, and Hsinchu were found to be toxic. The most toxic goby was *Y. nebulosus*, 93% of which were toxic. Specimens collected from Pingtung Prefecture were most toxic, the toxicity was more than 1000 MU/specimen. A seasonal variation of toxicity in *Y. nebulosus* was found, and the highest toxicity was observed for specimens collected in winter with a value of 4998 MU/specimen. The toxin was partially purified from the methanolic extract of each toxic species by Diaflo YM-1 membrane ultrafiltration and Bio-Gel P-2 column chromatography. High-performance liquid chromatography and electrophoresis analyses indicated that each toxic species contained tetrodotoxin (TTX) and anhydrotetrodotoxin. Among three toxic gobies, *P. palynema* and *R. caninus* are first reported to contain TTX.

KEY WORDS: **goby, *Prachaeturichtys palynema*, *Radigobius caninus*, tetrodotoxin, toxicity, *Yongeichthys nebulosus*.**

INTRODUCTION

The toxicity of gobies has been studied since the 1970s. Noguchi and Hashimoto[1] demonstrated that a goby *Gobius criniger* (also known as *Ctenogobius criniger*, *Yongeichthys nebulosus* or *Rhinogobius nebulosus*) collected in Amami-Oshima Island showed a distinct regional variation of toxicity. In the toxic specimens, the skin was generally high in toxicity. Furthermore, previous studies[2,3] identified the toxin as tetrodotoxin. In 1977, Elam *et al.*[2] also found that three species of gobies, *Clevelandia ios*, *Acanthogobius flavimanus*, and *Gillichthys mirabilis*, collected from California coast contained neurotoxins. The tetrodotoxin-like toxin in *G. mirabilis* was found principally in the ovaries and liver.

In Taiwan, 87 species of gobies have been recorded.[5] These gobies mainly distribute in estuaries, downstream portions of rivers, and coasts in Taiwan. They are very easily caught and consumed by local people. Yang[6] reported several outbreaks of fatal poisoning in southern Taiwan due to ingestion of toxic gobies. However, no data about toxicity and toxins were available. Recently, several paralytic food poisoning incidents due to ingestion of *Y. nebulosus* occurred in southern and northern Taiwan.[7,8] In the meantime, some toxic marine animals such as xanthid crabs, gastropods, and starfish collected from southern Taiwan were found to contain tetrodotoxin (TTX) and paralytic shellfish poison (PSP).[9–13] Although *G. criniger* collected from Amami-Oshima Island was reported to contain only TTX, the toxic component in specimens collected from Taiwan was not identified.[1] To establish the hygienic data of Taiwanese gobies, further intensive investigation of toxic gobies was performed. Toxins of toxic gobies were also purified and analyzed for composition.

MATERIALS AND METHODS

Materials

Three hundred and five specimens of 12 goby species (Table 1) were collected from estuaries, downstream portions of rivers, and coasts of southern, western, and northern Taiwan (Fig. 1) from August 1996 to July 1998.

*Corresponding author: Tel: 886-2-24622192. Fax: 886-2-24626602. Email: b0064@notu66.ntou.edu.tw

Received 20 January 1999. Accepted 16 December 1999.

Table 1 Gobies collected from August 1996 to July 1998 in Taiwan

Species	Specimen no.	Body weight (g)	Body length (cm)	Place of collection[2]
Bolephthalmus chinensis	10	44.6 ± 9.1[1]	15.6 ± 1.5	C
Cryptocentrus filiter	6	13.8 ± 3.1	12.7 ± 1.1	Ka
Yongeichthys nebulosus	112	12.2 ± 5.6	9.7 ± 2.4	(M)[3], (Pe), (Pi), I
Gobius sp.	16	31.0 ± 12.5	14.4 ± 2.6	H, I, Pi
Periophalmus contonensis	20	1.6 ± 1.0	6.1 ± 1.8	M, T
Prachaeturichtys palynema	60	5.8 ± 1.6	9.7 ± 0.9	(Ka)
Radigobius caninus	12	13.0 ± 4.9	10.8 ± 1.9	(Ka)
Rhinogobius brunneu	2	1.4 ± 1.0	5.1 ± 0.9	M
R. candidianus	15	2.3 ± 1.3	5.5 ± 1.0	Ke, M
R. gigus	16	4.0 ± 2.5	6.2 ± 1.3	I
R. giurinus	10	1.2 ± 0.2	4.8 ± 0.4	M
Rhinogobius sp.	26	2.0 ± 2.0	4.6 ± 1.3	I

[1] Mean ± SD.
[2] C, Chiayi Prefecture; H, Hsinchu City; I, Ilan Prefecture; Ka, Kaohsiung Prefecture; Ke, Keelung City; M, Miaoli Prefecture; Pe, Penghu Prefecture; Pi, Pingtung Prefecture; T, Taipei Prefecture.
[3] Parentheses represent the place where toxic specimens were collected.

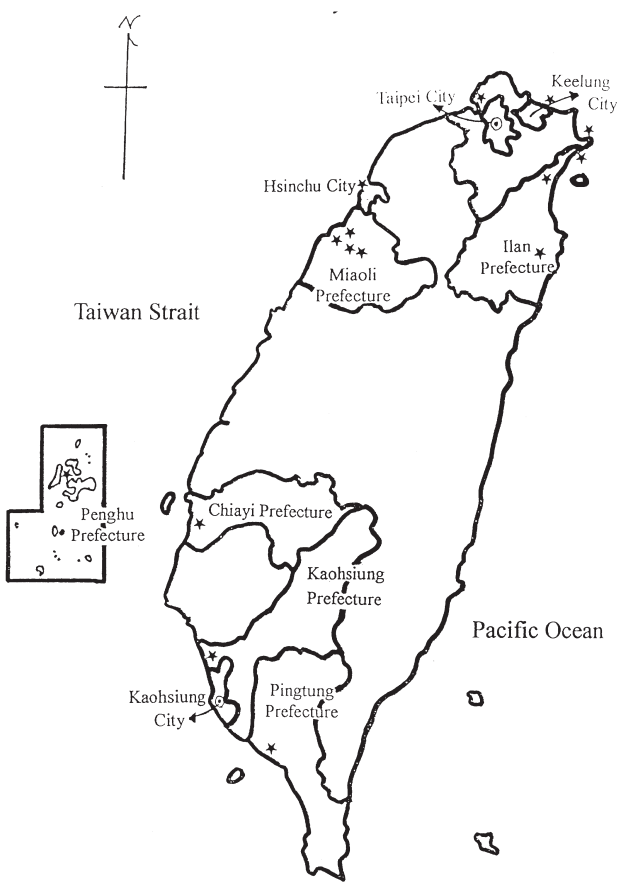

Fig. 1 A map of Taiwan showing the collection sites (*).

The specimens were transported to the laboratory of the National Taiwan Ocean University, Keelung, ROC, and kept frozen at −20°C until use.

The Institute of Cancer Research (ICR) strain mice were purchased from the National Laboratory Animal Breeding and Research Center, Taipei, ROC. Authentic TTX and anhydrotetrodotoxin (anh-TTX), which were obtained from the liver of the puffer *Takifugu oblongus*, were used as the reference standards.[14,15] Authentic gonyautoxins 1-4 (GTX 1-4) and saxitoxins (STX) obtained from the purple clam *Soletellina diphos* and the xanthid crab *Zosimus aeneus*, respectively, were also used as the reference standards.[16,17]

Assay for toxicity

The frozen specimens were partially thawed and dissected into muscle, skin, fin, head, viscera and gonad just before the toxicity assay. Each dissected tissue was weighed, homogenized with three volumes of 1% acetic acid in methanol for 5 min, and centrifuged (2000 g, 20 min). The operation was repeated twice. The supernatants were combined, concentrated under reduced pressure at 45°C, and examined for toxicity by the standard mouse assay for TTX.[18,19] Toxicity was expressed in mouse units. Due to the standard deviation (SD) of toxicity score sometimes being larger than mean value, the standard error ($SE^2 = SD^2/n$) was used for toxicity data. One mouse unit (MU) is defined as the amount of toxin required to kill a 20 g ICR strain male mouse in 30 min after a single intraperitoneal injection.

Purification of goby toxin

After the toxicity assay, the extracts of toxic species were combined, concentrated under reduced pressure at 45°C, and defatted with dichloromethane. The aqueous layer was concentrated and filtered through a Diaflo YM-1 membrane (Amicon, Beverly, MA, USA) to remove substances of more than 1000 daltons. The filtrate was applied to a Bio-Gel P-2 column (2 × 94 cm; Bio-Rad, Hercules, CA, USA) which was developed with 0.03 M acetic acid. Toxic fractions were combined, freeze-dried, dissolved in a small amount of water, and submitted to the analyses described below.

Electrophoresis

Electrophoresis was performed in 5 × 18 cm cellulose acetate strips (Chemetron, Milano, Italy) in 0.08 M Tris-HCl buffer (pH 8.7; Sigma, St Louis, USA) under a constant current of 0.8 mA/cm for 1 h. The TTX was viewed as yellow or blue fluorescent spots under a UV lamp (365 nm) after spraying the strip with 10% KOH and heating at 110°C for 10 min. The GTX and STX were also viewed as green and blue fluorescent spots after spraying the other strip with 1% H_2O_2 and under the same UV wavelength and heating conditions as TTX were treated.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was performed on a reversed-phase column (Merck Lichrospher 100 RP-18, 4 mm I.D. × 20 cm; E. Merck, Darmstadt, Germany). The mobile phase for TTX and GTX analysis was sodium 1-heptane sulfonate (2 mM) in methanol (1%)-potassium phosphate buffer (0.05 M, pH 7.0). For STX analysis, the mobile phase was 1-heptane sulfonate (2 mM) in methanol (20%)-potassium phosphate buffer (0.05 M, pH 7.0). The TTX was detected by mixing the eluate with 3 N NaOH at a ratio of 1:1, followed by heating at 99°C for 0.4 min, and monitoring the fluorescence at 505 nm with 381 nm excitation. In the case of GTX and STX analysis, the eluates were mixed with an equal volume of periodate reagent. The periodate reagent was composed of equal volume of A solution (1.8 N KOH:2.5 M ammonia formate:formanide = 1:4:5) and B solution (0.08 M periodic acid solution). The fluorogenic reaction was performed at 65°C for 0.7 min. The intensity of the fluorescence was measured at 388 nm with 344 nm excitation.[20]

RESULTS

Toxicity of goby

As shown in Fig. 1, 305 specimens of gobies were collected from 15 locations of nine cities and prefectures in northern, western, and southern Taiwan. As summarized in Table 1, 10 species were certainly identified, while identification of the other two species was made to a genus level. Among them, three toxic species, *Yongeichthys nebulosus*, *Prachaeturichtys palynema*, and *Radigobius caninus*, which had medium body size, were found in Miaoli, Penghu, Pingtung, and Kaohsiung Prefectures. As shown in Table 2, the frequencies of toxic specimens for *Y. nebulosus*, *P. palynema*, and *R. caninus*, were 93, 20, and 50%, respectively. For *Y. nebulosus*, the total toxicity was 2142 ± 21 (mean ± SE) MU/specimen ranging from ND to 8060 MU/specimen. The average toxicities of the tissues were 220 ± 2 MU/g in fin, followed by 205 ± 2 MU/g in head, 169 ± 2 MU/g in viscera, 162 ± 1 MU/g in gonad, 139 ± 2 MU/g in muscle, and 137 ± 2 MU/g in skin. *Radigobius caninus* was less toxic, its average total toxicity being 30 ± 6 MU/specimen ranging from ND to 49 MU/specimen. The toxicity was found in every tissue except for the fin. *Prachaeturichtys palynema* contained the least amount of toxins which were distributed mainly in the head and muscle.

The regional variation of toxicity in *Y. nebulosus* is shown in Table 3. The frequencies of toxic specimens of *Y. nebulosus* in Penghu, Pingtung, and Miaoli Prefecture were 100, 98 and 88%, respectively. The toxicity of *Y. nebulosus* specimens, which were collected from Pingtung Prefecture, was the highest, ranging from ND to 8060 (2379 ± 24) MU/specimen. *Yongeichthys nebulosus* specimens collected from Penghu and Miaoli Prefecture had smaller body size and showed less toxicity ranging

Table 2 Anatomical distribution of toxicity in toxic goby species

Species	Toxic ratio (%)	Anatomic toxicity (MU/g)						Total toxicity (MU/ specimen)[5]
		Muscle	Skin	Fin	Head	Viscera	Gonad	
Yongeichthys nebulosus	93 (104/112)[2]	ND-684[1] (139 ± 21)[3]	ND-648 (137 ± 11)	ND-1020 (220 ± 21)	ND-855 (205 ± 21)	ND-1045 (169 ± 21)	ND-553 (162 ± 21)	0–8060 (2142 ± 222)
Prachaeturichtys palynema	20 (12/60)	ND-52 (7 ± 7)	ND	ND	ND-88 (9 ± 7)	ND	–[4]	0–18 (8 ± 7)
Radigobius caninus	50 (6/12)	ND-5 (2 ± 3)	ND-8 (3 ± 3)	ND	ND-10 (4 ± 3)	ND-30 (12 ± 14)	ND-18 (8 ± 7)	0–49 (30 ± 21)

[1] ND means less than 4 MU/g.
[2] Toxic specimens/total specimens.
[3] Mean ± SE calculated on the assumption that the toxicity scores of all non-toxic specimens were zero.
[4] Not assayed.
[5] The toxicity scores of all non-toxic specimens were zero.

Table 3 Regional variations of toxicity in *Yongeichthys nebulosus*

Place of collection	Body length (cm)[1]	Body weight (g)[1]	Toxic ratio (%)	Total toxicity (MU/specimen)
Ilan Prefecture	9.7 ± 1.8	7.3 ± 2.6	0 (0/5)[3]	0[2]
Miaoli Prefecture	5.6 ± 1.1	2.1 ± 1.3	88 (7/8)	0–172 (78 ± 23)[4]
Penghu Prefecture	4.0 ± 1.0	1.0 ± 0.9	100 (10/10)	10–82 (32 ± 9)
Pingtung *Prefecture*	10.4 ± 1.4	13.4 ± 4.3	98 (87/89)	0–8060 (2379 ± 227)

[1] Mean ± SD.
[2] The toxicity scores of all non-toxic specimens were zero.
[3] Toxic specimens/total specimens.
[4] Mean ± SE.

from 10 to 82 (32 ± 3) and from ND to 172 (78 ± 8) MU/specimen, respectively. However, *Y. nebulosus* specimens collected from Ilan Prefecture, which is located in northern Taiwan, were non-toxic.

The seasonal variation of toxicity in *Y. nebulosus* collected at Tungkang, southern Taiwan is shown in Table 4. The total toxicity of *Y. nebulosus* was found to be much higher in winter and early spring. The average toxicities were 3323 ± 104, 4998 ± 190, 4143 ± 197, and 2217 ± 107 MU in December, January, February, and March 1998, respectively.

Toxin composition

Figure 2 shows HPLC chromatograms of the extracts of *Y. nebulosus*, *P. palynema*, and *R. caninus* together with that of the authentic TTX. Each extract gave rise to two peaks whose retention times coincided well with those of TTX and anh-TTX. However, peaks corresponding to authentic GTX and STX were not found in all extracts. Electrophoresis of the extracts showed at least two spots (data not shown). Among them, two spots were indistinguishable from TTX and anh-TTX, respectively, both in migration distances (8.0 and 5.5 cm) and in fluorescent colors (yellow and blue). However, no spots of GTX and STX were observed. Each extract (50 MU/5 mL) was further heated with concentrated sulfuric acid and found to be non-toxic. This chemical character was the same as that of TTX.[14]

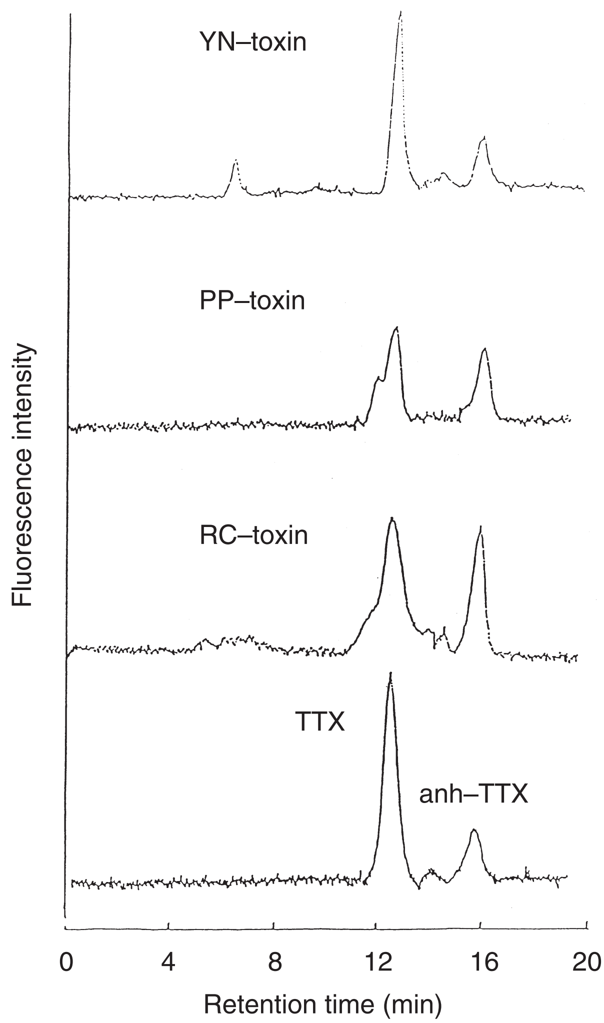

Fig. 2 HPLC chromatograms of the extracts of *Yongeichthys nebulosus* (YN-toxin), *Prachaeturichtys palynema* (PP-toxin), and *Radigobius caninus* (RC-toxin), along with authentic tetrodotoxin (TTX) and anh-TTX.

DISCUSSION

In this study we showed that three species of Taiwanese gobies, *Y. nebulosus*, *P. palynema*, and *R. caninus*, contained TTX. Among them, *Y. nebulosus* is well known to be toxic, and the other two species, *P. palynema* and *R. caninus*, were first reported as toxic. These toxic gobies were mainly from southern and western Taiwan. In these

Table 4 Seasonal and anatomical variation of toxicity in *Yongeichthys nebulosus* collected at Tungkang, Pingtung Prefecture

Date of collection	Specimen no.	Body length[1] (cm)	Body weight[1] (g)	Anatomic toxicity[2] (MU/g)						Total toxicity[2]	
				Muscle	Skin	Fin	Head	Viscera	Gonad	(MU/specimen)	(MU/g)
Aug. 1996	10	9.9 ± 0.9	8.0 ± 1.3	81 ± 19	–[3]	67 ± 13	40 ± 9	ND[4]	ND	410 ± 101	49 ± 9
Mar. 1997	10	9.6 ± 0.6	11.6 ± 2.5	359 ± 51	281 ± 54	466 ± 85	409 ± 70	490 ± 82	–	4600 ± 532	410 ± 54
Oct. 1997	10	9.8 ± 2.1	11.4 ± 2.3	16 ± 3	101 ± 9	70 ± 13	108 ± 16	88 ± 16	ND	718 ± 76	63 ± 6
Dec. 1997	10	10.3 ± 1.3	15.0 ± 3.0	208 ± 41	147 ± 32	290 ± 51	334 ± 63	109 ± 28	87 ± 22	3323 ± 329	222 ± 16
Jan. 1998	10	11.6 ± 1.2	18.8 ± 2.0	132 ± 47	119 ± 25	346 ± 63	381 ± 70	269 ± 51	299 ± 63	4998 ± 601	260 ± 25
Feb. 1998	10	10.1 ± 1.6	11.7 ± 3.2	307 ± 35	298 ± 57	501 ± 76	312 ± 47	290 ± 44	100 ± 41	4143 ± 623	338 ± 32
Mar. 1998	10	9.7 ± 0.6	12.0 ± 1.5	93 ± 9	96 ± 25	156 ± 19	198 ± 38	161 ± 32	159 ± 32	2217 ± 339	193 ± 32
May 1998	7	10.3 ± 0.8	12.1 ± 1.6	9 ± 3	11 ± 3	10 ± 9	14 ± 9	24 ± 16	21 ± 16	136 ± 47	11 ± 6
Jun. 1998	6	11.8 ± 0.5	19.5 ± 4.0	47 ± 9	48 ± 6	69 ± 9	52 ± 13	85 ± 25	–	956 ± 146	53 ± 13
Jul. 1998	6	11.7 ± 1.3	18.1 ± 3.4	8 ± 6	7 ± 6	24 ± 9	8 ± 3	46 ± 54	–	152 ± 95	9 ± 6

[1] Mean ± SD.
[2] Mean ± SE calculated on the assumption that the toxicity scores of all non-toxic specimens were zero. Data of March 1997 were cited from Lin *et al.*[8]
[3] – No data.
[4] ND means less than 4 MU/g.

areas, several outbreaks of fatal poisoning occurred[6–8] and several TTX-bearing animals and TTX-producing bacteria were recently found to be present.[21–27]

We also found that the major toxic species was *Y. nebulosus*, eliciting the highest frequency of toxic specimens. The toxicity was high in fins and head, intermediate in viscera and gonad, and low in muscle and skin. These results coincided well with those obtained by Noguchi and Hashimoto[1] who indicated that the toxicity of *Y. nebulosus* was generally high in fins and head.

The study on the regional variation of toxicity in *Y. nebulosus* showed that the frequencies of toxic specimens collected in Pingtung, Penghu, and Miaoli Prefecture were much higher than that in Ilan. Moreover, the average total toxicity of the specimens in Pingtung Prefecture was 30–74 times higher than those in the other areas where toxic specimens were found. This may indicate that the goby could be easily toxicified in southern Taiwan. The seasonal variation of toxicity in *Y. nebulosus* collected in Pingtung Prefecture was obvious. Much higher total toxicity was found in winter and early spring.

The toxins of these toxic gobies were all composed of TTX and anh-TTX. The toxin composition of *Y. nebulosus* was the same as that of previous reports.[2–4,8] Interestingly, this toxin composition was also the same as those of *Babylonia formosae*,[28] *Natica vitellus*, and *Polinices didyma*[22] collected from the same coastal area of Pingtung Prefecture. However, the toxin of some marine animals, such as the gastropod *N. clathrata* and *N. lineata*,[9–10] the xanthid crab *Z. aeneus*, *Atergatis floridus*, and *Demania reynaudi*,[11–12] and the starfish *Astropecten scoparius*,[13] which were collected in the same area, were composed of both TTX and PSP toxins. The reason for a different toxin composition in these toxic marine animals should be further studied.

ACKNOWLEDGMENTS

This study was supported by Department of Health, Republic of China (Grant No. DOH-86-TD-089). The authors thank Dr T. Noguchi and Dr O. Arakawa, Nagasaki University, Japan, for their useful advice.

REFERENCES

1. Noguchi T, Hashimoto Y. Toxicity of the goby, *Gobius criniger*. *Nippon Suisan Gakkaishi* 1971; **37**: 642–647.
2. Elam K-S, Fuhrman F-A, Kim Y-H, Mosher H-S. Neurotoxins from three species of California goby: *Clevelandia ios*, *Acanthogobius flavimanus*, and *Gillichthys mirabilis*. *Toxicon* 1977; **15**: 45–49.
3. Hashimoto Y, Noguchi T. Occurrence of a tetrodotoxin-like substance in a goby *Gobius criniger*. *Toxicon* 1971; **9**: 79–84.
4. Noguchi T, Hashimoto Y. Isolation of tetrodotoxin from a goby *Gobius criniger*. *Toxicon* 1973; **11**: 305–308.
5. Shen S-C. *Fishes of Taiwan*. National Taiwan University, Taipei, 1993.

6. Yang H-C. Poisonous and venomous fishes of Taiwan. *Annu. Rep. Sci. Taiwan Museum* 1967; **10**: 36–71.
7. Lin S-J, Cheng C-A, Tsai Y-H, Sa C-H, Deng J-F, Hwang D-F. Food poisoning due to ingestion of goby *Yongeichthys nebulosus* Forsskal. *J. Food Drug Anal.* 1996; **4**: 359–364.
8. Lin S-J, Chen J-B, Hsu K-T, Hwang D-F. Acute goby poisoning in southern Taiwan. *J. Nat. Toxins* 1999; **8**: 141–147.
9. Hwang D-F, Cheng C-A, Jeng S-S. Gonyautoxin-3 as a minor toxin in the gastropod *Niotha clathrata* in Taiwan. *Toxicon* 1994; **32**: 1573–1579.
10. Cheng C-A, Lin S-J, Hwang D-F. Paralytic toxins of the gastropod *Natica lineata* in Pingtung Prefecture. *Food Sci.* 1996; **23**: 845–853.
11. Hwang D-F, Tsai Y-H, Chai T, Jeng S-S. Occurrence of tetrodotoxin and paralytic shellfish poison in Taiwan crab *Zosimus aeneus*. *Fisheries Sci.* 1996; **62**: 500–501.
12. Tsai Y-H, Hwang D-F, Chai T, Jeng S-S. Toxicity and toxic components of two xanthid crabs *Atergatis floridus* and *Demania reynaudi* in Taiwan. *Toxicon* 1997; **35**: 1327–1335.
13. Lin S-J, Tsai Y-H, Lin H-P, Hwang D-F. Paralytic toxins in Taiwanese starfish *Astropecten scoparius*. *Toxicon* 1998; **36**: 799–803.
14. Goto T, Kishi Y, Takahashi S, Hirata Y. Tetrodotoxin. *Tetrahedron* 1965; **21**: 2059–2088.
15. Hwang D-F, Noguchi T, Arakawa O, Abe T, Hashimoto K. Toxicological studies on several species of puffer in Taiwan. *Nippon Suisan Gakkaishi* 1988; **54**: 2001–2008.
16. Hwang D-F, Noguchi T, Nagashima Y, Liao I-J, Hashimoto K. Occurrence of paralytic shellfish poison in the purple clam *Soletellina diphos* (bivalve). *Nippon Suisan Gakkaishi* 1987; **53**: 623–626.
17. Daigo K, Uzu A, Arakawa O, Noguchi T, Seta H, Hashimoto K. Isolation and some properties of neosaxitoxin from a xanthid crab *Zosimus aeneus*. *Nippon Suisan Gakkaishi* 1985; **51**: 309–313.
18. Yasumoto Y. Tetrodotoxin. In: Environmental Health Bureau, Ministry of Health and Welfare (ed.). *Standard Methods of Analysis in Food Safety Regulation-Chemistry*. Japan Food Hygiene Association, Tokyo. 1991; 296–300.
19. Hwang D-F, Jeng S-S. Bioassay of tetrodotoxin by using ICR strain male mouse. *J. Chin. Biochem. Soc.* 1991; **20**: 80–86.
20. Nagashima Y, Maruyama J, Noguchi T, Hashimoto K. Analysis of paralytic shellfish poison and tetrodotoxin by ion-pairing high performance liquid chromatography. *Nippon Suisan Gakkaishi* 1987; **53**: 819–823.
21. Hwang D-F, Chueh C-H, Jeng S-S. Occurrence of tetrodotoxin in the gastropod mollusk *Natica lineata* (lined moon shell). *Toxicon* 1990; **28**: 21–27.
22. Hwang D-F, Tai K-P, Chueh C-H, Lin L-C, Jeng S-S. Tetrodotoxin and derivatives in several species of the gastropods Naticidae. *Toxicon* 1991; **29**: 1019–1024.
23. Hwang, D-F, Lu S-C, Jeng S-S. Occurrence of tetrodotoxin in the gastropods *Rapana rapiformis* and *R. venosa venosa*. *Mar. Biol.* 1991; **111**: 655–659.
24. Hwagn D-F, Lin L-C, Jeng S-S. Occurrence of tetrodotoxin-related toxins in the gastropod mollusk *Niotha clathrata* from Taiwan. *Nippon Suisan Gakkaishi* 1992; **58**: 63–67.
25. Hwang D-F, Lin L-C, Jeng S-S. Occurrence of a new toxin and tetrodotoxin in two species of the gastropod mollusk Nassariidae. *Toxicon* 1992; **30**: 41–46.
26. Hwang D-F, Cheng C-A, Chen H-C *et al.* Microflora and tetrodotoxin-producing bacteria in the lined moon shell *Natica lineata*. *Fisheries Sci.* 1994; **60**: 567–571.
27. Cheng C-A, Hwang D-F, Tsai Y-H *et al.* Microflora and tetrodotoxin-producing bacteria in a gastropod, *Niotha clathrata*. *Food Chem. Toxicol.* 1995; **33**: 929–934.
28. Lin S-J, Liao H-J, Hwang DF. Toxicity of gastropods *Babylonia formosae* and *Charonia sauliae* in Taiwan. *J. Nat. Toxins* 1996; **5**: 305–313.