

Occurrence of CFP and Development of CTXs Analysis Method in Japan

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Ciguatera Fish Poisoning: CFP

Ciguatera is ...

- the disease caused by ingestion of tropical finfish.
- prevalent in tropical and sub-tropical Pacific and Indian Oceans and Caribbean Sea.
- derived from Caribbean name 'cigua' for shellfish *Turbo pica*.
- most frequent seafood poisoning in the world.
 - 20,000 – 50,000 patients, annually
- rarely fatal.
- persistent for months or years in severe case.
- caused by ciguatoxins.

Symptoms

- Incubation period ----- 1-36 hours (mostly within 24 h)
- Gastrointestinal ----- early stage
 - vomiting, diarrhea, nausea, abdominal pain
- Neurological ----- most common
 - dry ice sensation ----- “paradoxical” perception or reversal of temperature perception to cold stimulus
 - paresthesia of extremities and circumoral region
 - arthralgia, pruritus, rash.
- Cardiovascular ----- not common but severe
 - bradycardia, hypotension.
- most of the patients recover within 3 days
- malaise, paresthesia and pruritus may persist for weeks or even years in severe cases.

Accumulation of ciguatoxins

via food chain



dinoflagellate

G. toxicus



herbivorous
animals



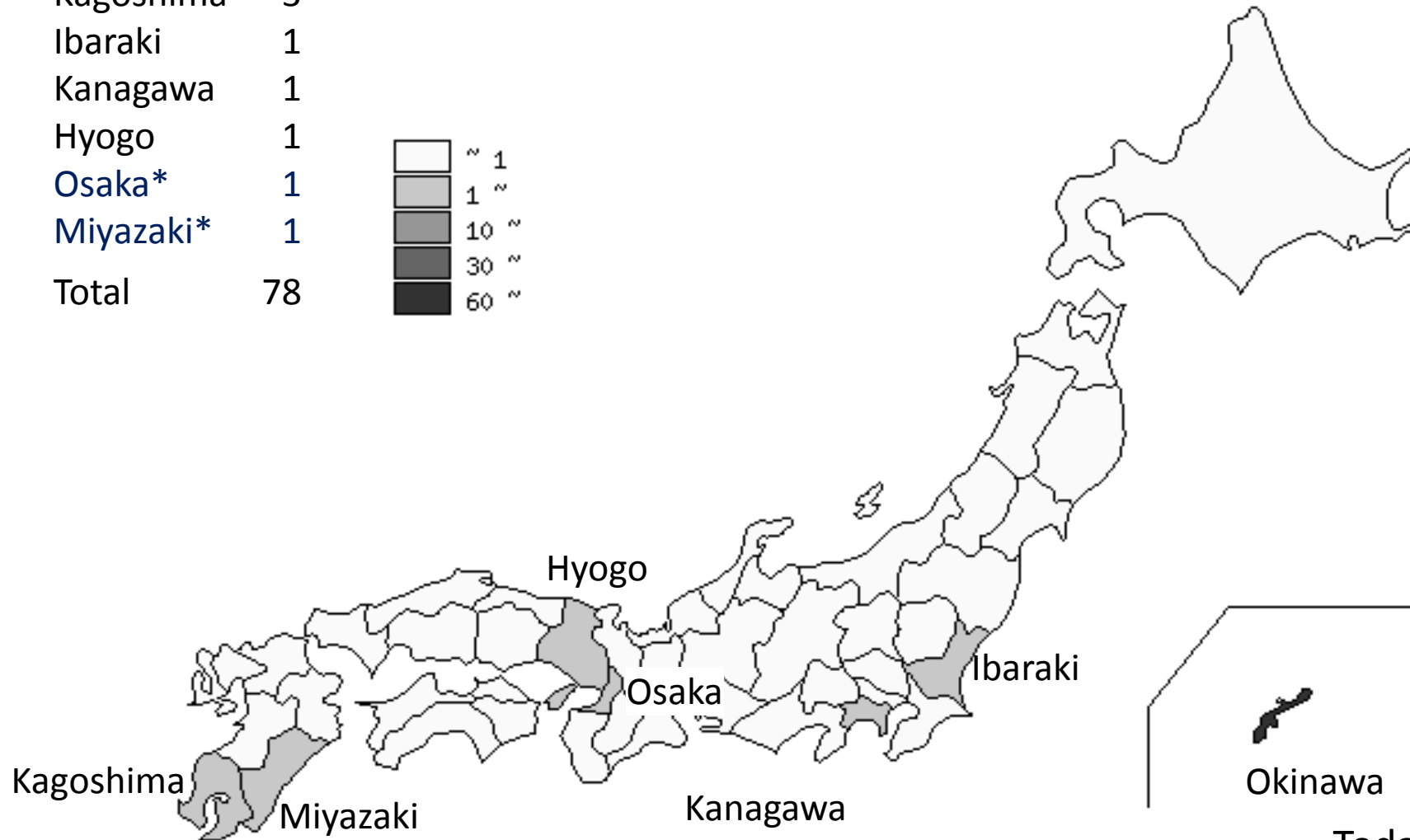
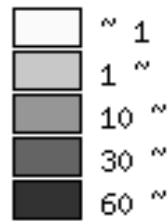
carnivorous fish

intoxication

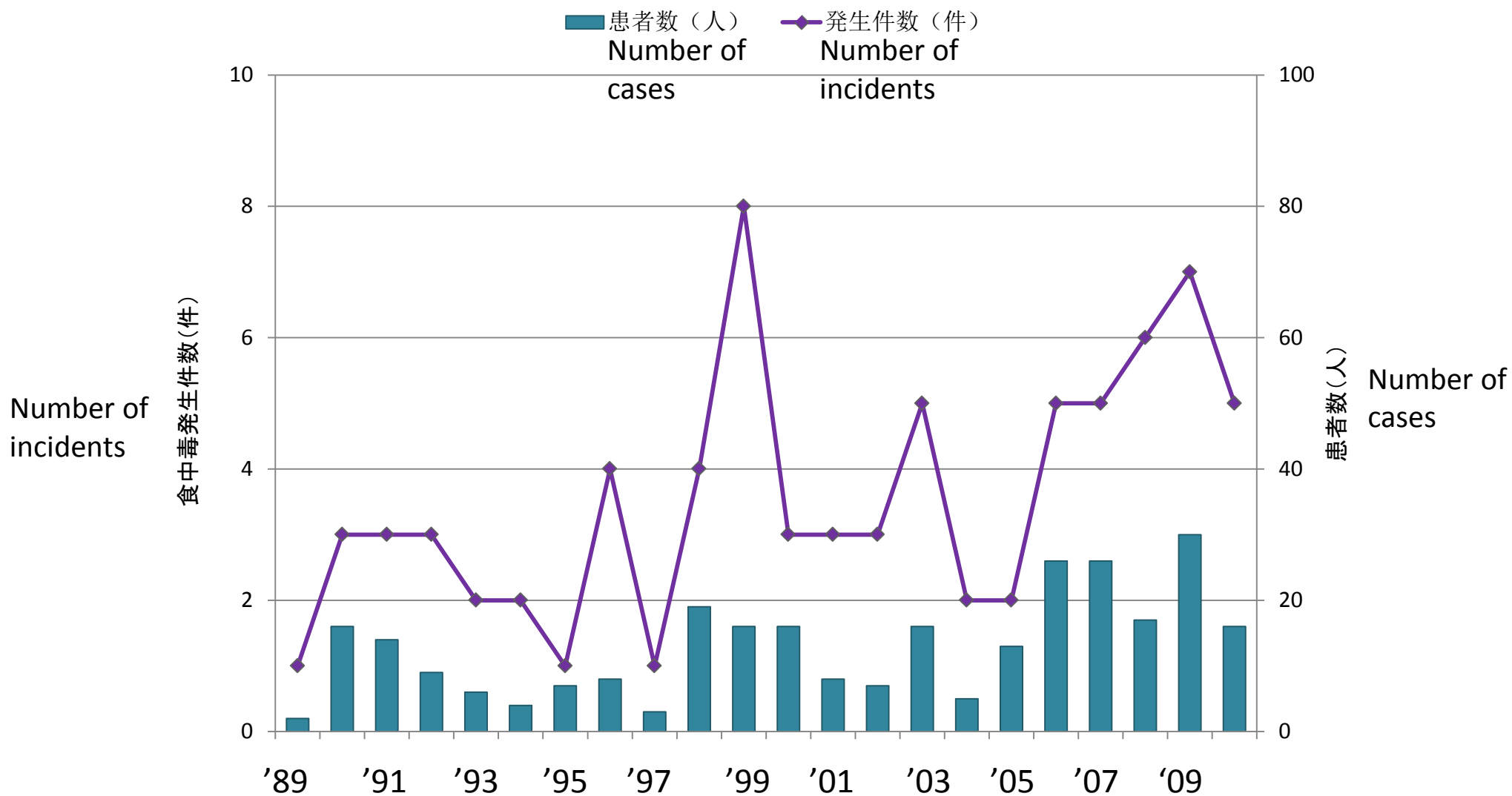
dry ice sensation

CFP incidents in Japan (1989-2010)

Okinawa	70
Kagoshima	3
Ibaraki	1
Kanagawa	1
Hyogo	1
Osaka*	1
Miyazaki*	1
Total	78



Toda et al., 2012



Number of incidents and cases of CFP in Japan (1989-2010)

Toda et al., 2012

Ciguatera Incidents in Okinawa

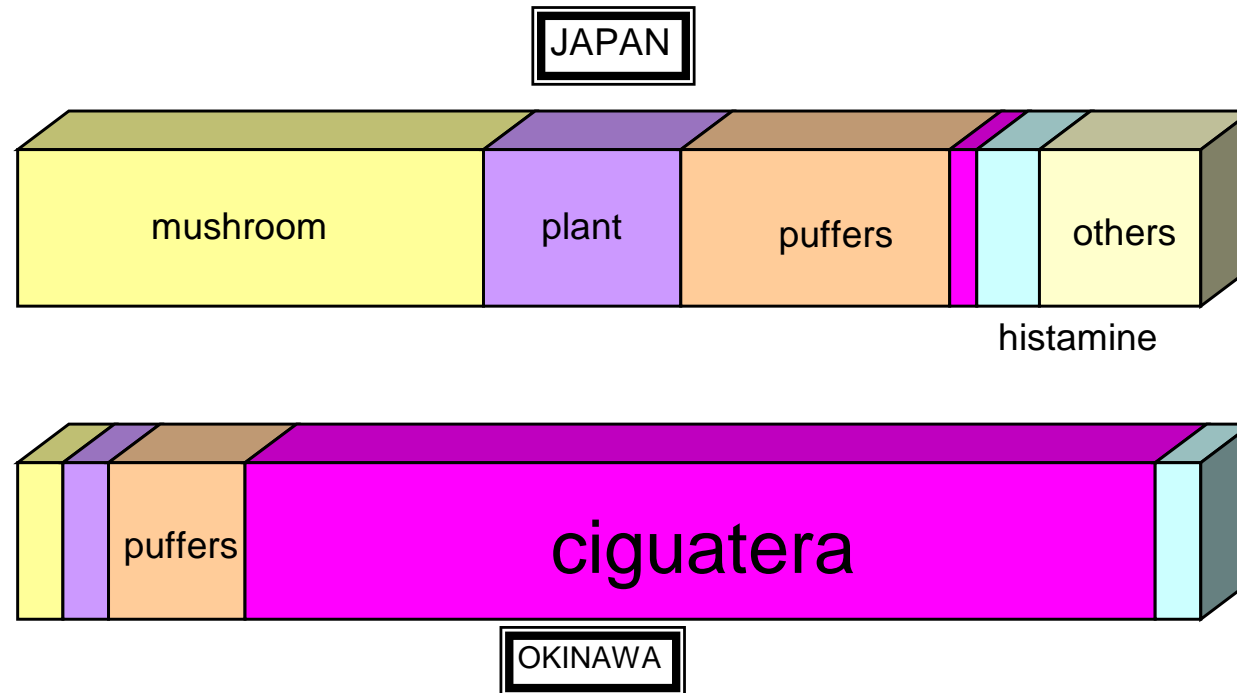


Okinawa, also known as Ryukyu, is located in the south-westernmost end of Japan, consisting of 48 islands.

Its climate is subtropical and It has been recognized as ciguatera endemic area.

The population of Okinawa is 1.3 million.

Natural toxin and chemical food poisoning in Okinawa



CFP accounted for 70% of chemical or natural toxin food poisoning in Okinawa.

Officially reported CFP in Okinawa, 1997-2006.

- 33 incidents were reported.

- Seasonality seems thin.

0.024 incidents/10,000 people/year

- Many cases might be remain unreported.

No.	Date	Number of patients	Number of people consumed	Attack rate (%)	fish species	Japanese name	toxicity MU/g
1	18/Jan/97	6	9	67	<i>L. monostigma</i>	イッテンフエダイ	n.t. ²
2	21/Sep/97	3	5	60	<i>Lutjanus</i> sp.	フエダイ類	0.29
3	17/May/98	3	7	43	<i>L. bohar</i>	バラフエダイ	n.t.
4	17/Jun/98	3	5	60	<i>V. louti</i>	バラハタ	0.1
5	25/Feb/99	1	1	100	<i>E. fuscoguttatus</i>	アカマダラハタ	n.t.
6	26/Feb/99	1	2	50	<i>E. fuscoguttatus</i>	アカマダラハタ	n.t.
7	08/May/99	1	7	14	<i>L. bohar</i>	バラフエダイ	n.t.
8	24/May/99	2	6	33	<i>L. bohar</i>	バラフエダイ	n.t.
9	03/Aug/99	1	4	25	<i>L. monostigma</i>	イッテンフエダイ	n.t.
10	31/Aug/99	3	3	100	<i>Caranx ignobilis</i>	ロウニンアジ	n.t.
11	21/Sep/99	6	6	100	unidentified	不明	n.t.
12	18/Oct/99	1	11	9	<i>L. bohar</i>	バラフエダイ	n.t.
13	16/Jan/00	7	7	100	<i>E. polyphkadion</i>	マダラハタ	0.05
14	20/May/00	5	5	100	<i>L. bohar</i>	バラフエダイ	n.t.
15	24/Jun/00	4	26	15	snapper	フエダイ類	n.t.
16	19/May/01	3	3	100	unidentified	不明	n.t.
17	31/Aug/01	3	3	100	<i>L. monostigma</i>	イッテンフエダイ	>0.2
18	21/Jun/02	2	5	40	<i>L. bohar</i>	バラフエダイ	n.t.
19	04/Nov/02	2	2	100	<i>P. areolatus</i>	オオアオノメアラ	n.t.
20	30/Nov/02	3	3	100	<i>L. monostigma</i>	イッテンフエダイ	>0.8
21	01/Apr/03	2	5	40	<i>O. punctatus</i>	イシガキダイ	n.t.
22	03/Jul/03	4	4	100	<i>L. monostigma</i>	イッテンフエダイ	0.2
23	22/Jul/03	3	3	100	<i>L. monostigma</i>	イッテンフエダイ	>0.2
24	06/Oct/03	4	4	100	<i>V. louti</i>	バラハタ	0.4
25	06/Mar/04	2	3	67	moray eel	ウツボ	n.t.
26	18/Nov/04	3	4	75	<i>V. louti</i>	バラハタ	>0.2
27	06/Jul/05	7	9	78	<i>V. louti</i>	バラハタ	n.t.
28	10/Sep/05	6	6	100	<i>V. louti</i>	バラハタ	0.1
29	24/Jan/06	2	2	100	<i>V. louti</i>	バラハタ	n.t.
30	18/Mar/06	2	2	100	<i>L. bohar</i>	バラフエダイ	n.t.
31	26/May/06	1	27	4	<i>L. bohar</i>	バラフエダイ	0.1
32	29/Jun/06	4	4	100	<i>V. louti</i>	バラハタ	0.05
33	31/Dec/06	3	8	38	<i>V. louti</i>	バラハタ	n.t.

Frequency of causative species in the CFP incidents in Okinawa.

species	number of outbreaks	ratio (%)
<i>Variola louti</i>	8	24
<i>Lutjanus bohar</i>	8	24
<i>L. monostigma</i>	6	18
<i>Epinephelus fuscoguttatus</i>	2	6
Lutjanidae	2	6
<i>Plectropomus areolatus</i>	1	3
<i>Oplegnathus punctatus</i>	1	3
<i>E. polyphkadion</i>	1	3
surgeonfish	1	3
moray eel	1	3
unidentified	2	6
total	33	



Variola louti



Lutjanus bohar



Lutjanus monostigma

Toxicity (MU/g) of the leftover meals and/or remnant fish.

No.	fish species	test sample	toxicity MU/g
2	<i>Lutjanus</i> sp.	cooked flesh	0.29
4	<i>V. louti</i>	raw flesh	0.1
13	<i>E. polyphkadion</i>	cooked flesh	0.05
		soup ¹	< 0.025
17	<i>L. monostigma</i>	cooked flesh	>0.2
20	<i>L. monostigma</i>	cooked flesh	>0.8
22	<i>L. monostigma</i>	raw flesh	>0.2
		mixed soup ²	0.025
23	<i>L. monostigma</i>	mixed soup ²	>0.2
24	<i>V. louti</i>	raw flesh	0.4
		mixed soup ²	0.1
26	<i>V. louti</i>	flesh ³	>0.2
	<i>V. louti</i>	flesh ³	0.1
28	<i>V. louti</i>	raw flesh	0.1
31	<i>L. bohar</i>	cooked flesh	0.1
32	<i>V. louti</i>	raw flesh	0.05

¹ flesh and bones were removed and remainig were mixed.

² bones were removed and remainig were mixed.

³ treated with boiling water

Toxicity (MU/g) of the leftover meals and/or remnant fish in 12 incidents.

No.	fish species	test sample	toxicity MU/g
2	<i>Lutjanus</i> sp.	cooked flesh	0.29
4	<i>V. louti</i>	raw flesh	0.1
13	<i>E. polyphkadion</i>	cooked flesh	0.05
		soup ¹	< 0.025
17	<i>L. monostigma</i>	cooked flesh	>0.2
20	<i>L. monostigma</i>	cooked flesh	>0.8
22	<i>L. monostigma</i>	raw flesh	>0.2
		mixed soup ²	0.025
23	<i>L. monostigma</i>	mixed soup ²	>0.2
24	<i>V. louti</i>	raw flesh	0.4
		mixed soup ²	0.1
26	<i>V. louti</i>	flesh ³	>0.2
	<i>V. louti</i>	flesh ³	0.1
28	<i>V. louti</i>	raw flesh	0.1
31	<i>L. bohar</i>	cooked flesh	0.1
32	<i>V. louti</i>	raw flesh	0.05

- **Lowest toxicity of fish flesh**
0.05 MU/g (No. 13 & 32)

¹ flesh and bones were removed and remainig were mixed.

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Toxicity (MU/g) of the leftover meals and/or remnant fish.

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		mixed soup ²	0.025
23	<i>L. monostigma</i>	mixed soup ²	>0.2
24	<i>V. louti</i>	raw flesh	0.4
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26	<i>V. louti</i>	flesh ³	>0.2
	<i>V. louti</i>	flesh ³	0.1
28	<i>V. louti</i>	raw flesh	0.1
31	<i>L. bohar</i>	cooked flesh	0.1
32	<i>V. louti</i>	raw flesh	0.05

¹ flesh and bones were removed and remainig were mixed.

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³ treated with boiling water

- **Lowest toxicity of fish flesh**
0.05 MU/g (No. 13 & 32)

- **Lowest toxicity of meal**
 ≤ 0.025 MU/g (No. 13 & 32)

Toxicity (MU/g) of the leftover meals and/or remnant fish.

No.	fish species	test sample	toxicity MU/g
2	<i>Lutjanus</i> sp.	cooked flesh	0.29
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23	<i>L. monostigma</i>	mixed soup ²	>0.2
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		mixed soup ²	0.1
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	<i>V. louti</i>	flesh ³	0.1
28	<i>V. louti</i>	raw flesh	0.1
31	<i>L. bohar</i>	cooked flesh	0.1
32	<i>V. louti</i>	raw flesh	0.05

¹ flesh and bones were removed and remainig were mixed.

² bones were removed and remainig were mixed.

³ treated with boiling water

- **Lowest toxicity of fish flesh**
0.05 MU/g (No. 13 & 32)

- **Lowest toxicity of meal**
≤ 0.025 MU/g (No. 13 & 32)



**• The maximum allowance level
should be set below 0.025 MU/g
(0.175 ng CTX1B/g)**

Toxicity (MU/g) of the leftover meals and/or remnant fish.

No.	fish species	test sample	toxicity MU/g
2	<i>Lutjanus</i> sp.	cooked flesh	0.29
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³ treated with boiling water

- **Lowest toxicity of fish flesh**
0.05 MU/g (No. 13 & 32)

- **Lowest toxicity of meal**
≤ 0.025 MU/g (No. 13 & 32)



• **The maximum allowance level should be set below 0.025 MU/g (0.175 ng CTX1B/g)**

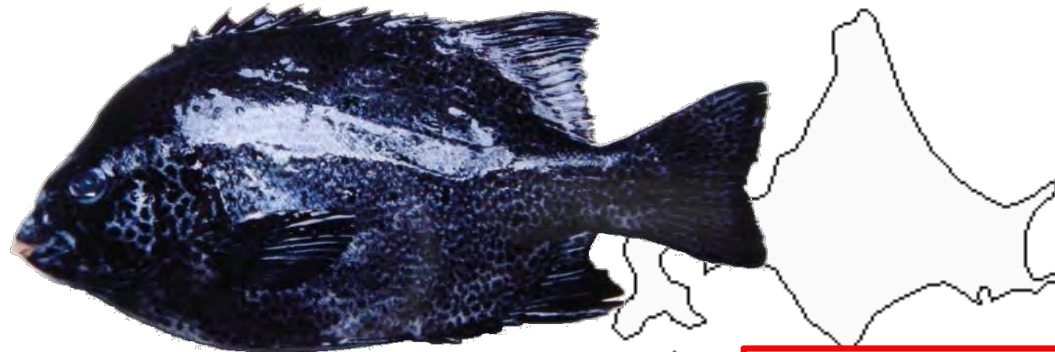
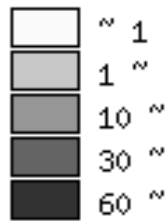
- **The minimum amount of consumption was estimated as 140 g (No. 28)**
0.1 MU/g x 140 g = 14 MU
= 98 ng CTX1B



CFP in Mainland Japan

CFP incidents in Japan (1989-2010)

Okinawa	70
Kagoshima	3
Ibaraki	1
Kanagawa	1
Hyogo	1
Osaka*	1
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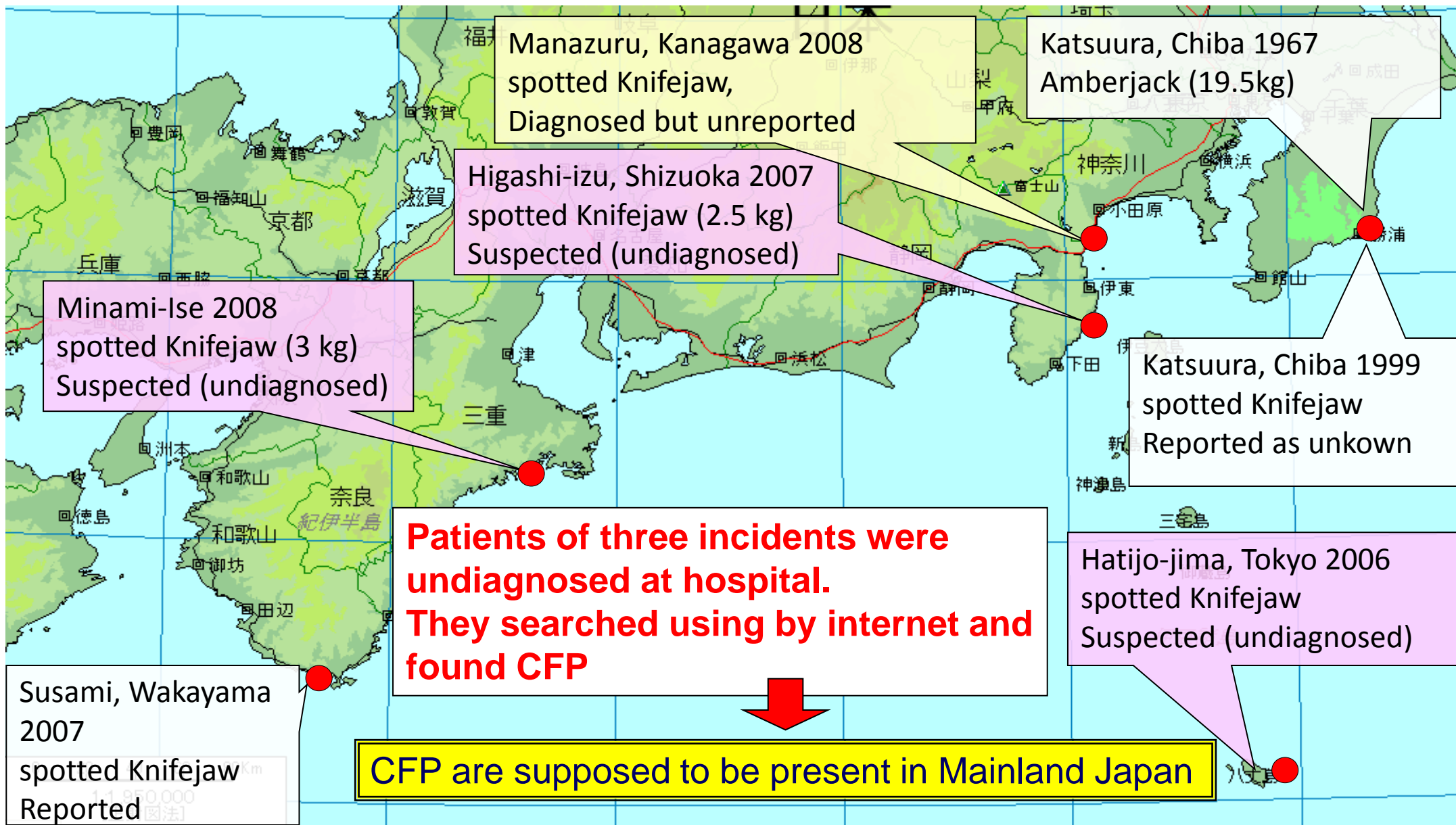
* Implicated fish was spotted Knifejaw, *Oplegnathus punctatus* collected off the mainland Japan.

An incident reported as unidentified is suspected to be CFP due to consumption of Spotted Knifejaw, *Oplegnathus punctatus* collected off Chiba.

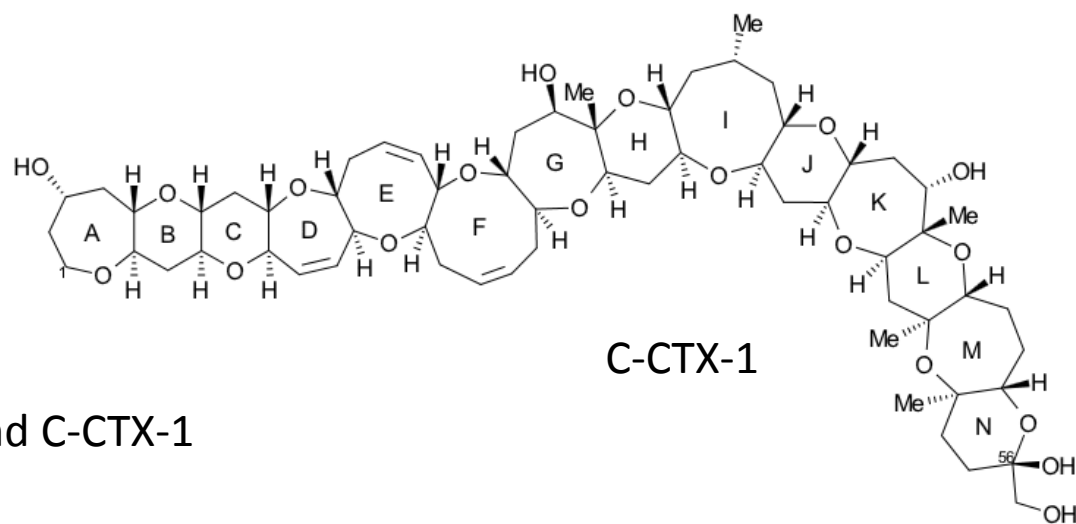
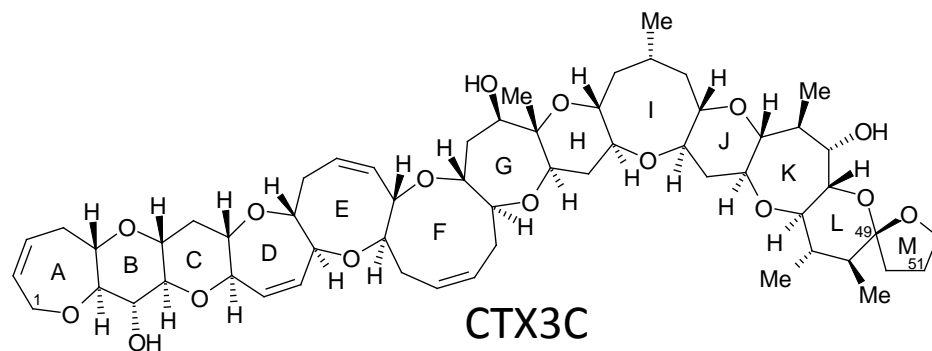
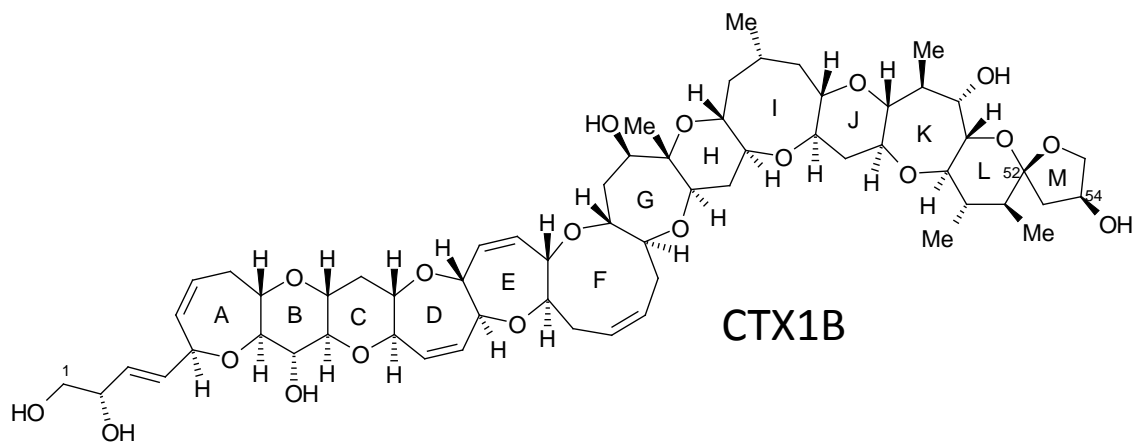


Toda et al., 2012

CFP in mainland Japan



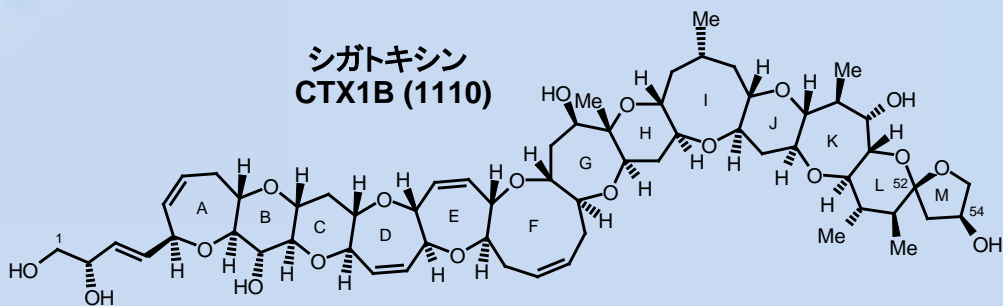
Development of CTXs Analysis Method in Japan



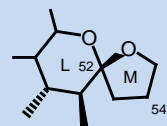
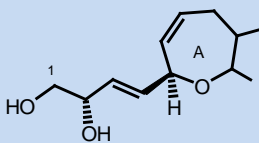
Structures of ciguatoxins, CTX1B, CTX3C and C-CTX-1

CTX1B congeners

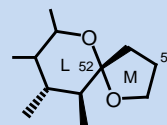
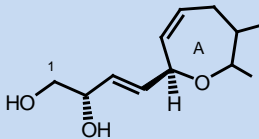
シガトキシン
CTX1B (1110)



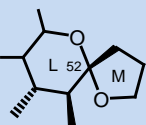
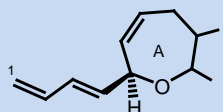
54-deoxyCTX1B
(1094)



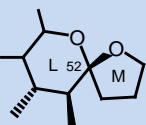
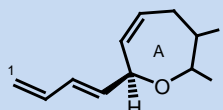
52-*epi*-54-deoxyCTX1B
(1094)



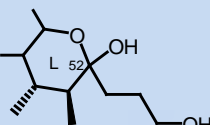
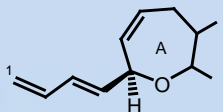
CTX4A
(1060)



CTX4B
(1060)

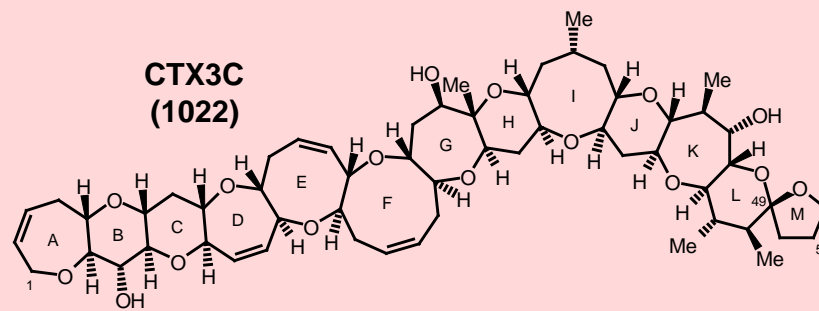


M-*seco*-CTX4A/B
(1078)

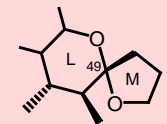
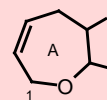


CTX3C congeners

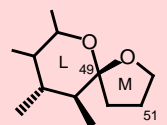
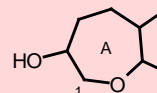
CTX3C
(1022)



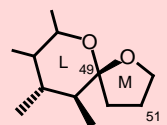
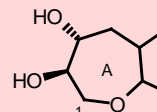
49-*epi*CTX3C
(1022)



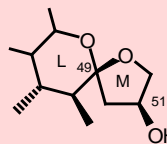
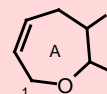
2-hydroxyCTX3C
(1040)



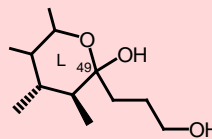
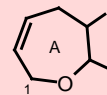
2,3-dihydroxyCTX3C
(1056)



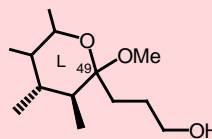
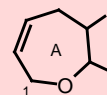
51-hydroxyCTX3C
(1038)



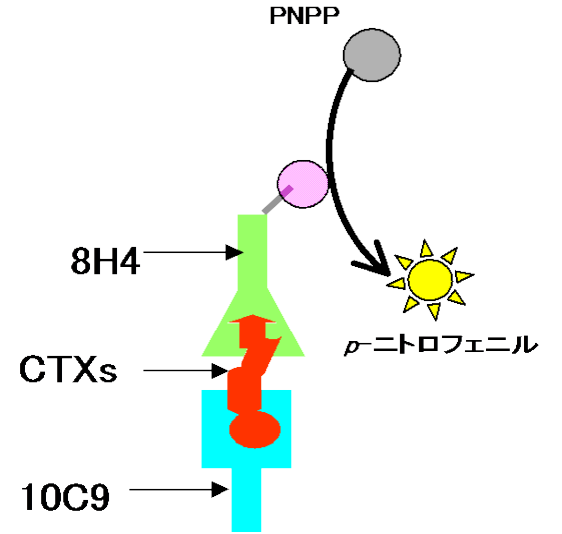
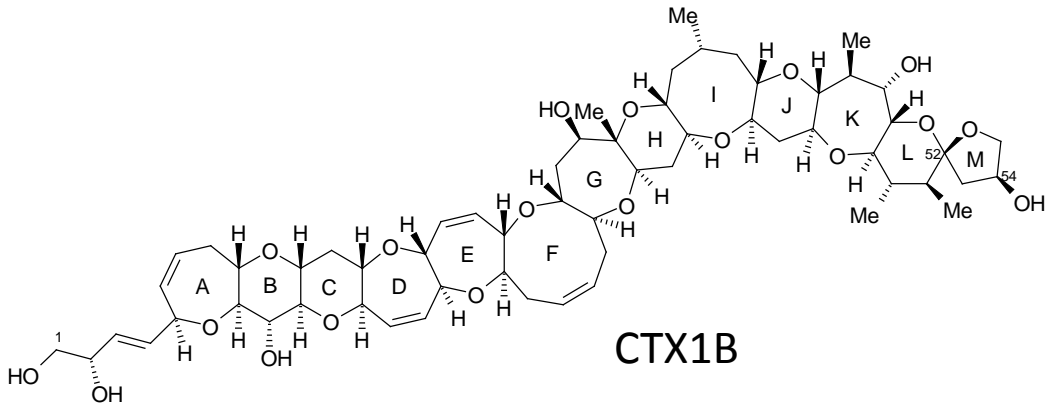
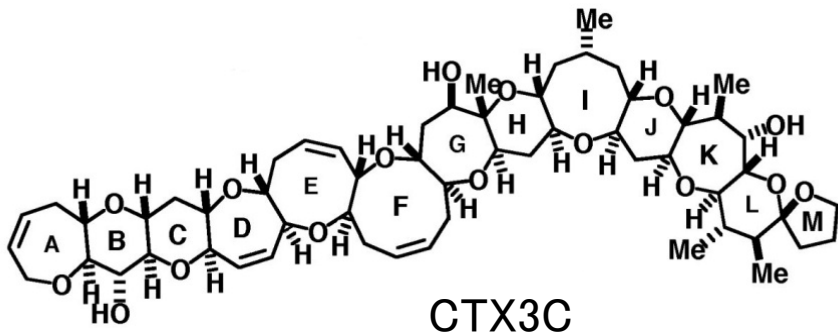
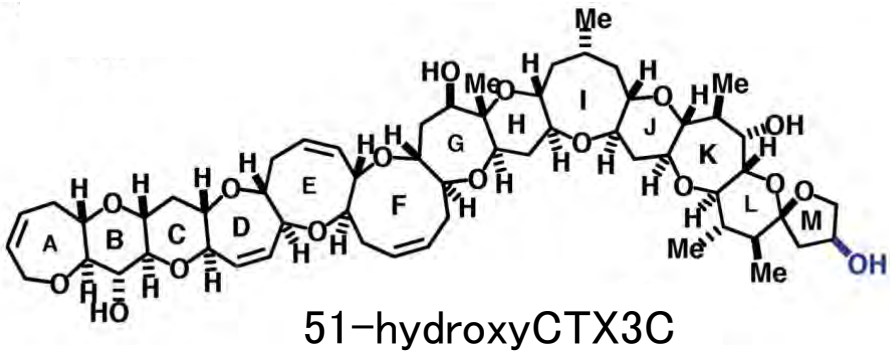
M-*seco*-CTX3C
(1040)



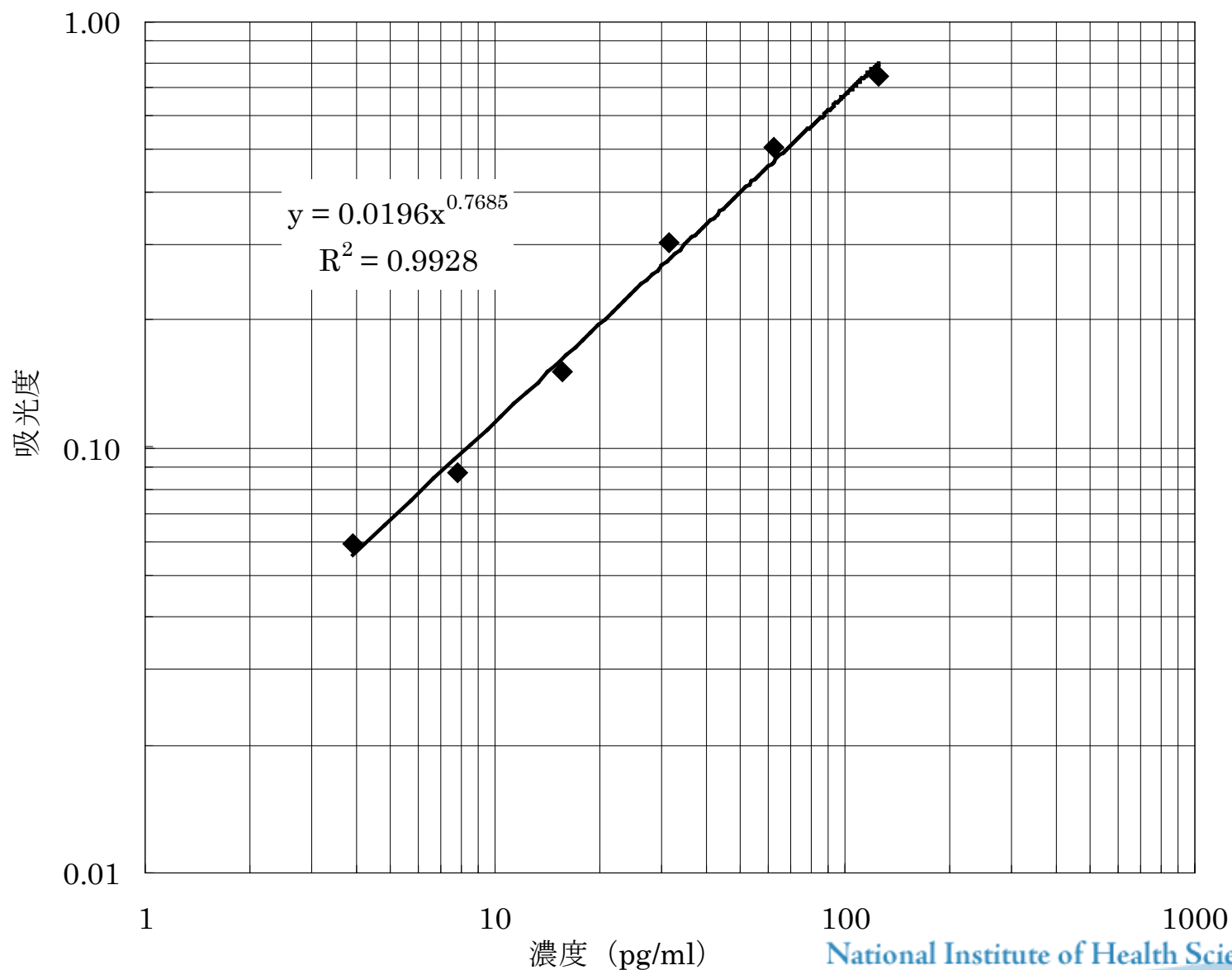
M-*seco*-CTX3C methyl acetal
(1054)



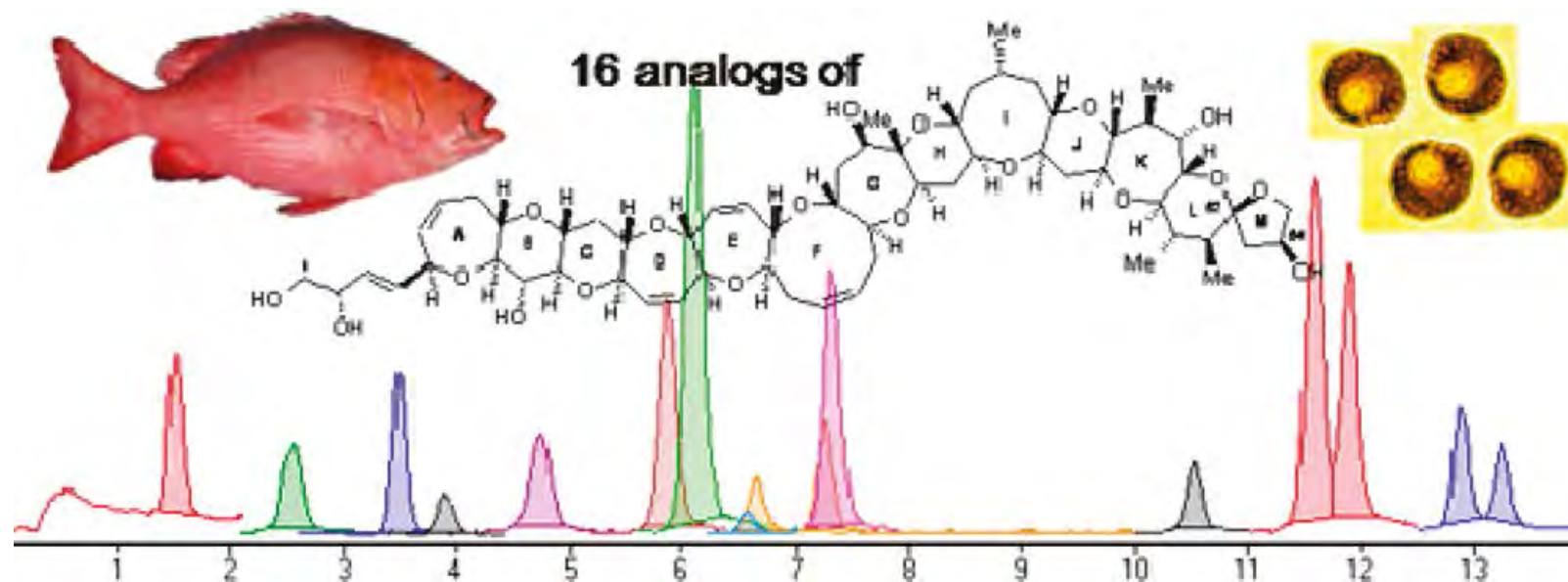
Detection of CTXs by ELISA



Calibration curve of 51-hydroxyCTX3C ELISA.



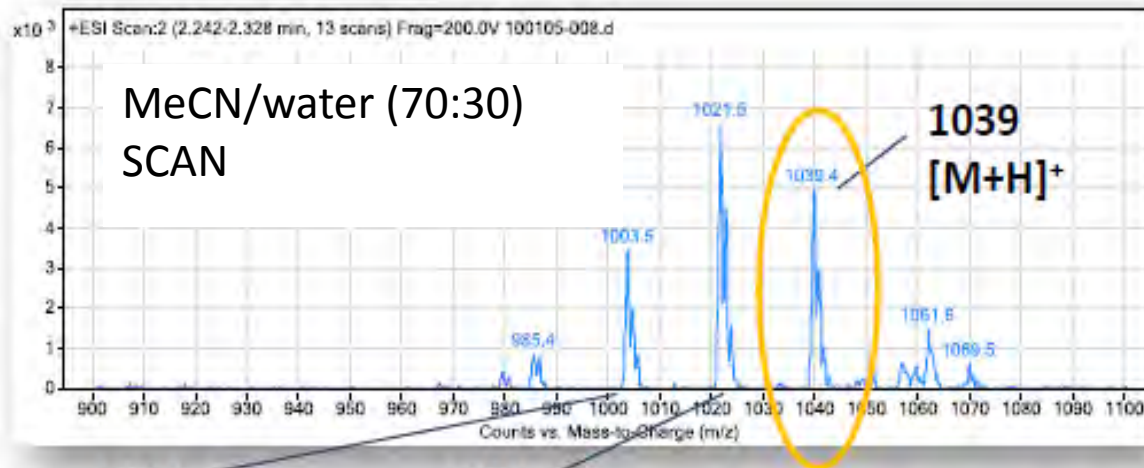
Detection of CTXs by LC-MS/MS



Mass spectrum of 51-hydroxyCTX3C.

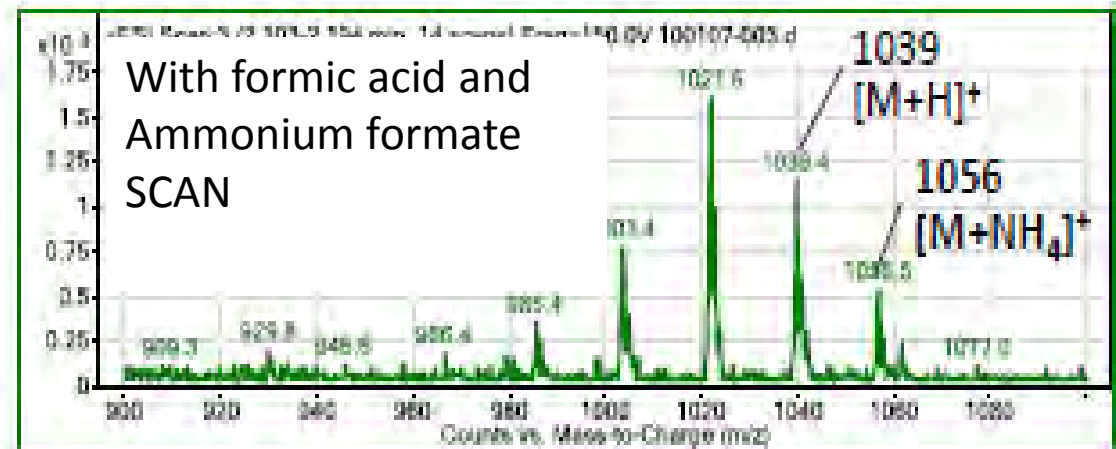
Many peaks are appeared using acetonitrile/water system as a eluent.

It may difficult to detect CTXs in low level.

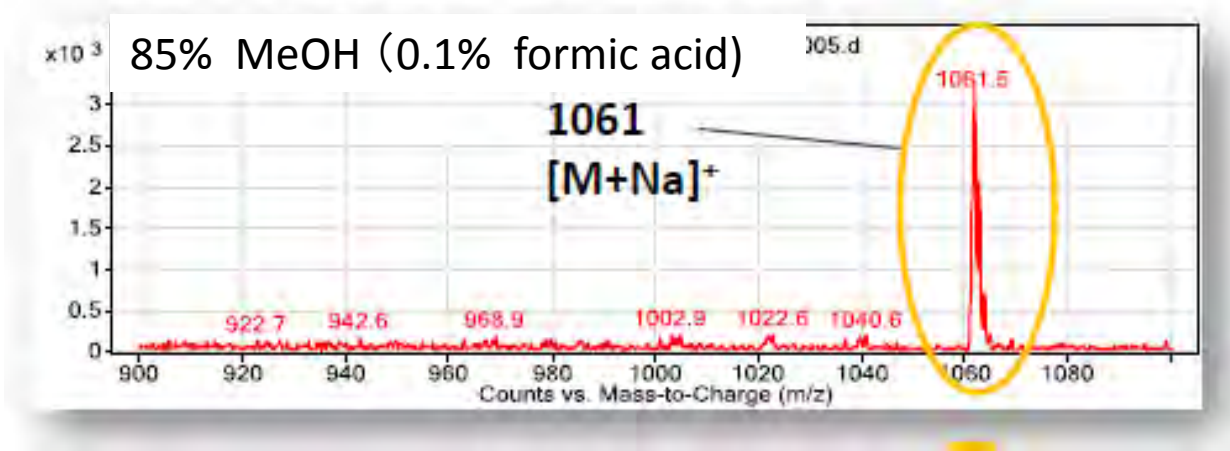


1003
[M+H-2H₂O]⁺

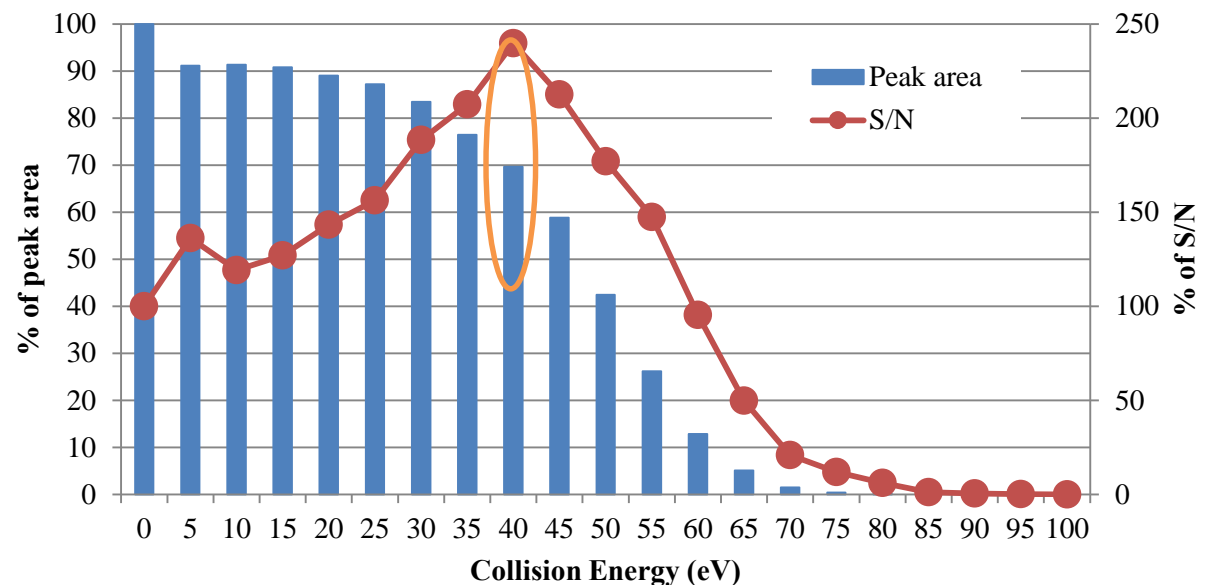
1021
[M+H-H₂O]⁺



Mass spectrum of 51-hydroxyCTX3C using MeOH/water system as eluent.
Only one peak $[M+Na]^+$ was detected.
But no fragment ion was detected in MS/MS.

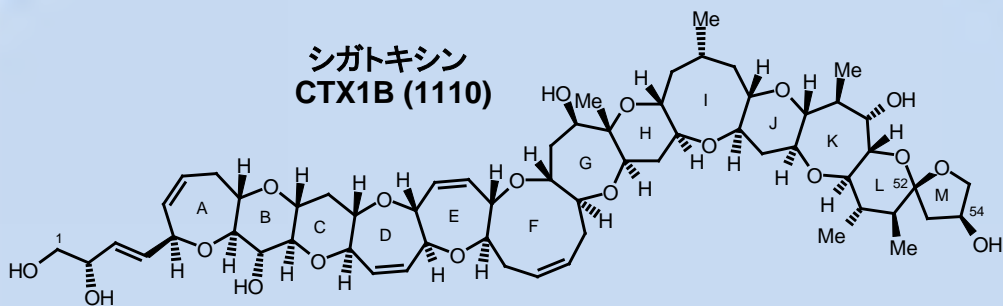


Mass transition : $[M+Na]^+ \rightarrow [M+Na]^+$
Collision energy was optimized for minimizing the interference from matrices

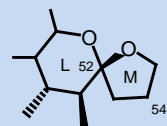
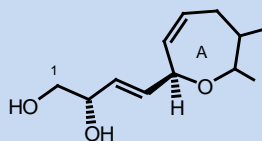


CTX1B congeners

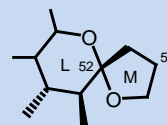
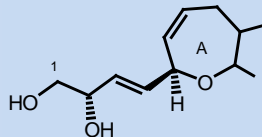
シガトキシン
CTX1B (1110)



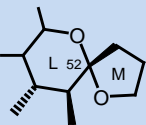
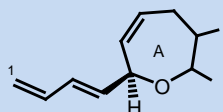
54-deoxyCTX1B
(1094)



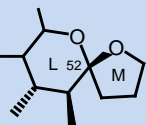
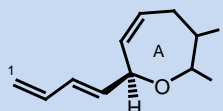
52-*epi*-54-deoxyCTX1B
(1094)



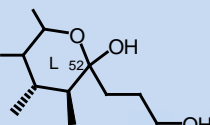
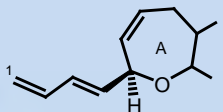
CTX4A
(1060)



CTX4B
(1060)

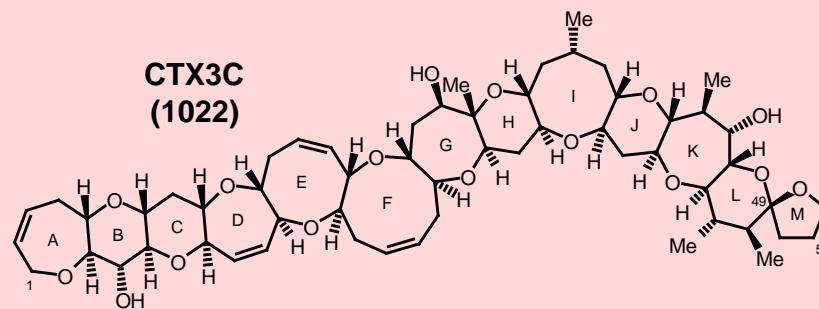


M-*seco*-CTX4A/B
(1078)

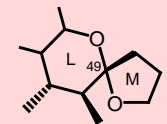
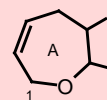


CTX3C congeners

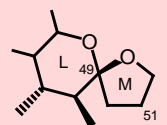
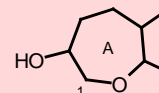
CTX3C
(1022)



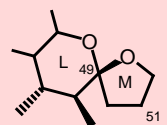
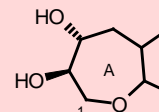
49-*epi*CTX3C
(1022)



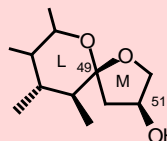
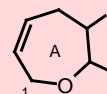
2-hydroxyCTX3C
(1040)



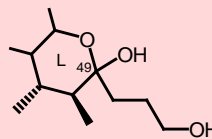
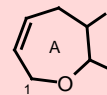
2,3-dihydroxyCTX3C
(1056)



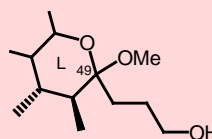
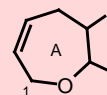
51-hydroxyCTX3C
(1038)



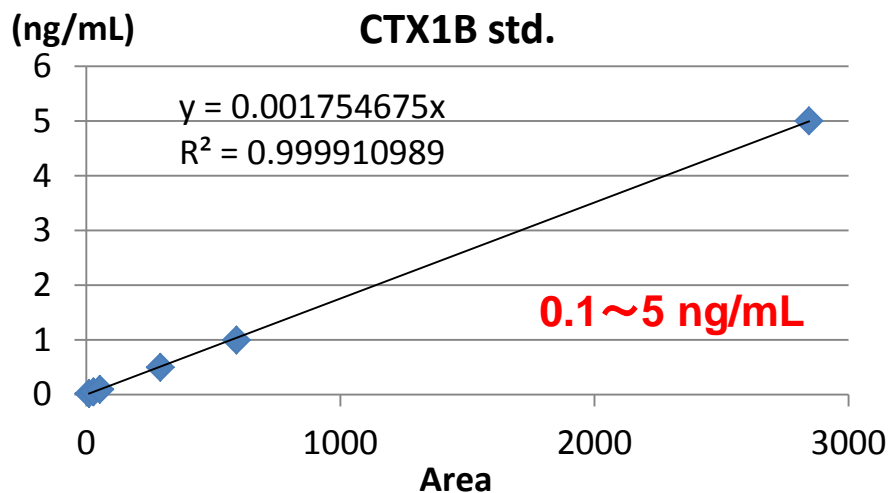
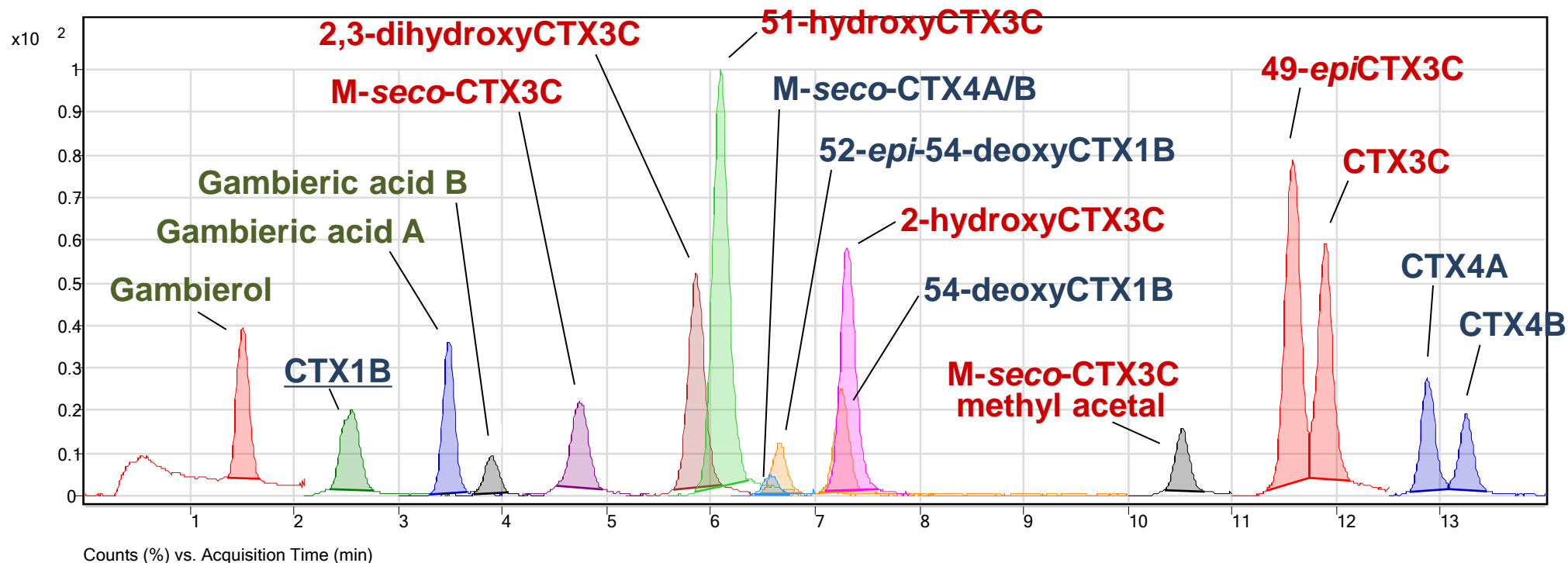
M-*seco*-CTX3C
(1040)



M-*seco*-CTX3C methyl acetal
(1054)



LC-MS/MS Chromatogram of CTXs standards.



CTX1B

Limit of Detection (S/N>3)

0.02 ng/mL (0.004 ng/g flesh)

Limit of Quantification (S/N>10)

0.1 ng/mL (0.02 ng/g flesh)

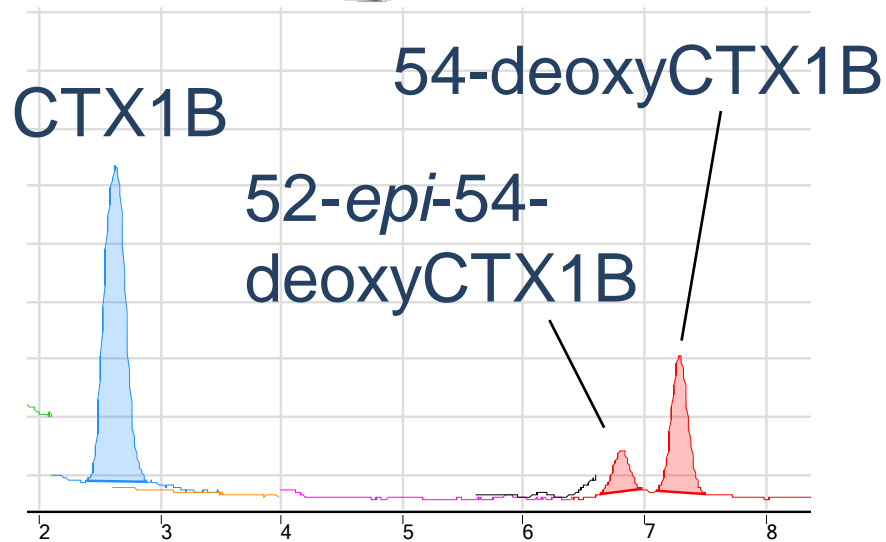
Toxin profiles of fish flesh implicated in CFP in Okinawa

snappers

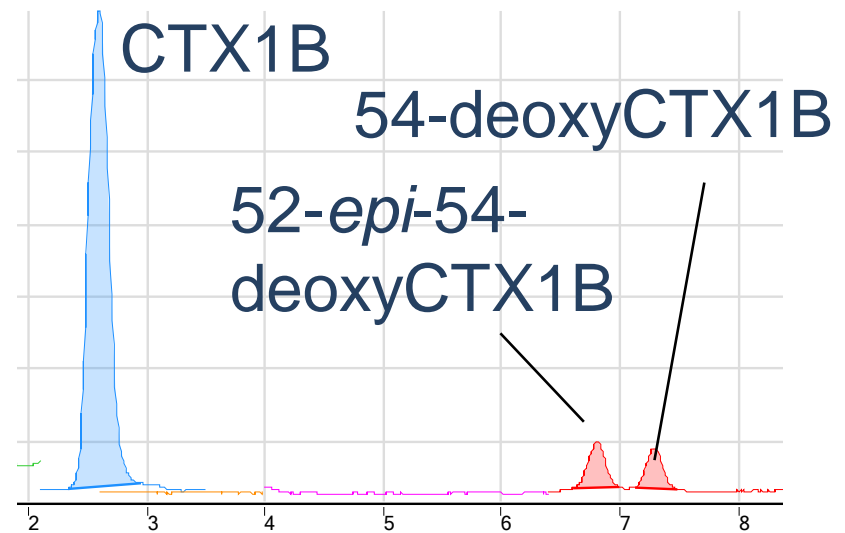
CTX1B congeners were detected.



L. bohar



L. monostigma



CTX1B is Account for majority of toxicity.
Deoxy-CTX1Bs are detected as minor toxins.

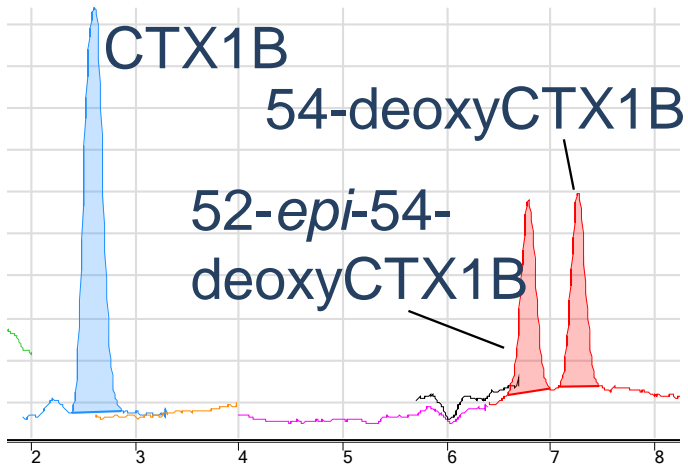
Toxin profiles of fish flesh implicated in CFP in Okinawa

Groupers

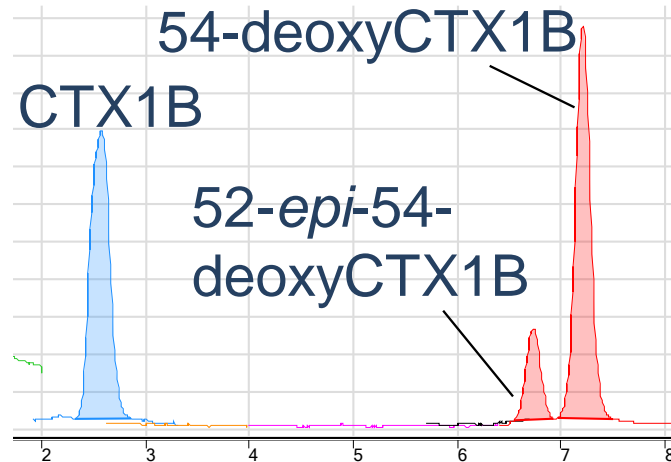
CTX1B congeners were detected.



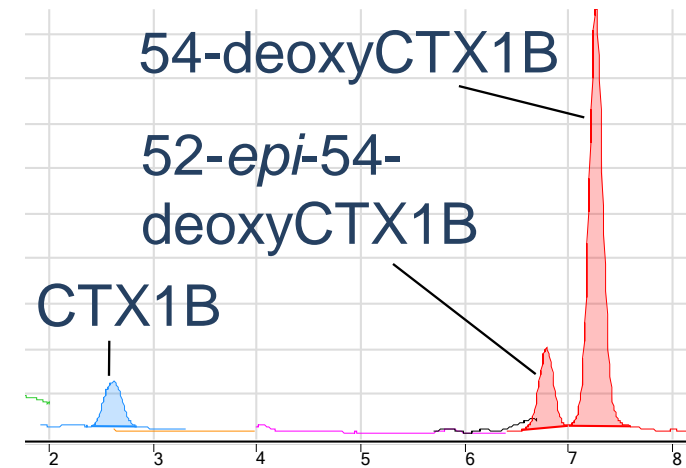
Anyperodon leucogrammicus



Variola louti



Plectropomus laevis

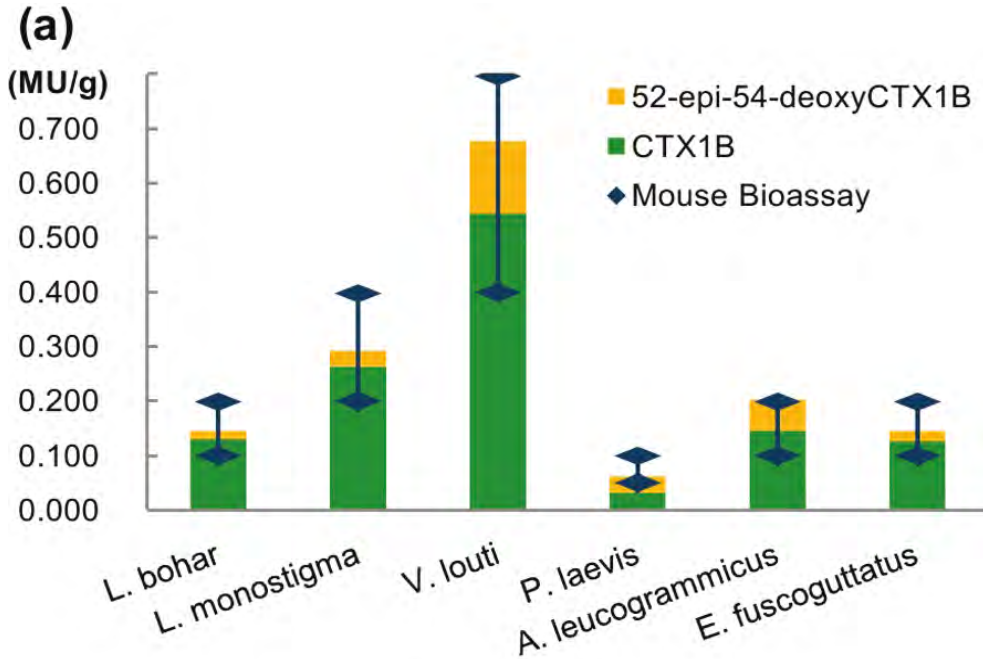


CTX1B is Account for majority of toxicity.

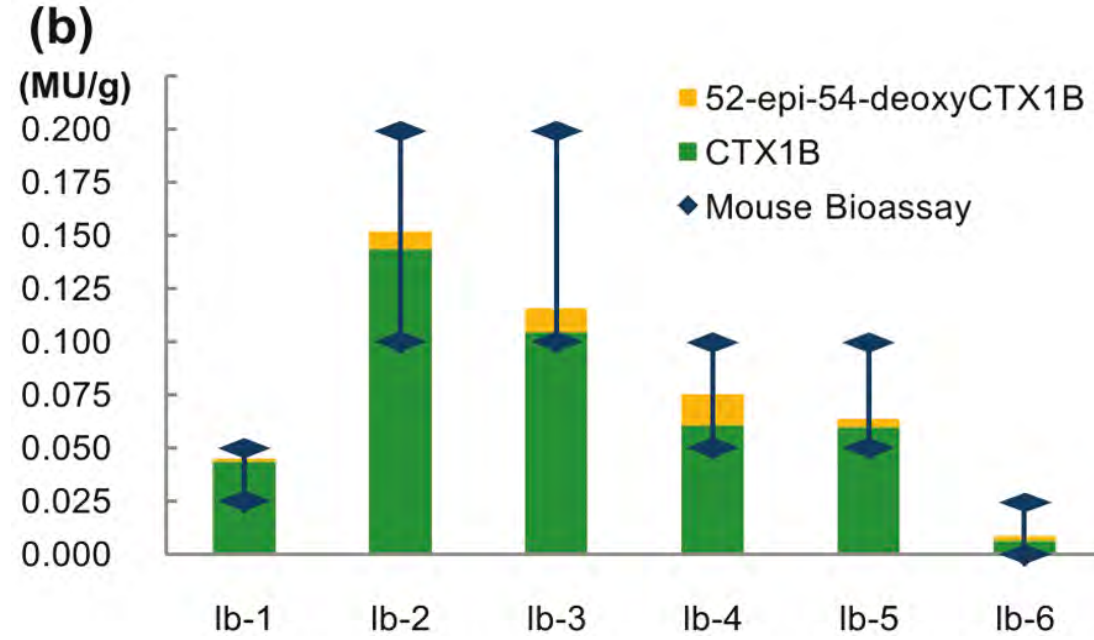
Significant amount of deoxy-CTX1Bs are detected.

In the case of *P. laevis*, 54-deoxyCTX1B is a major toxin.

Correlation between LC-MS/MS and MBA results

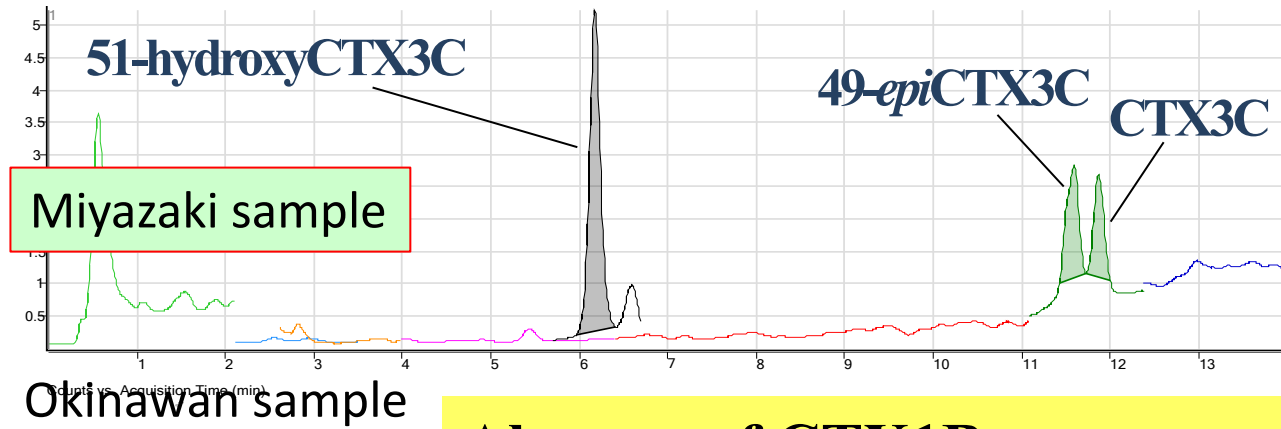


fish actually implicated in poisoning incidents

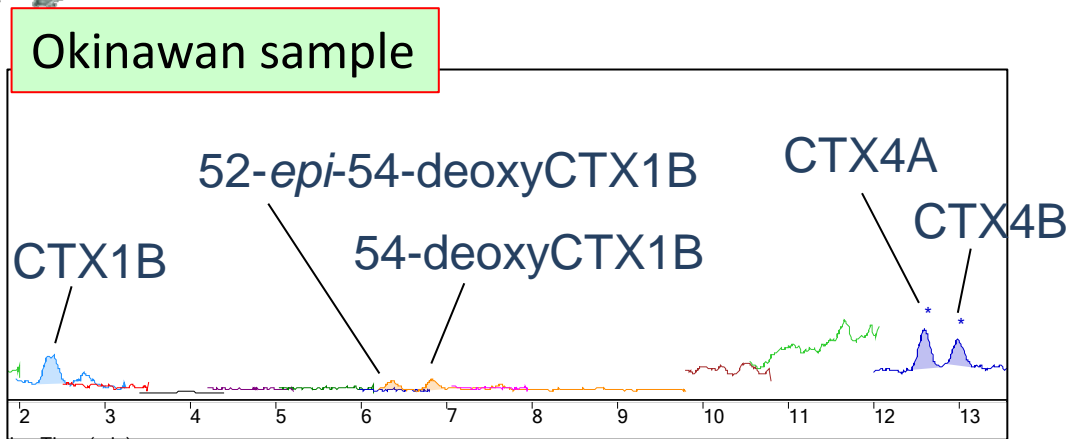


L. bohar tested by MBA in a monitoring study

Toxin profile of spotted knifejaw *Oplegnatus punctatus*.



Absence of CTX1B congeners



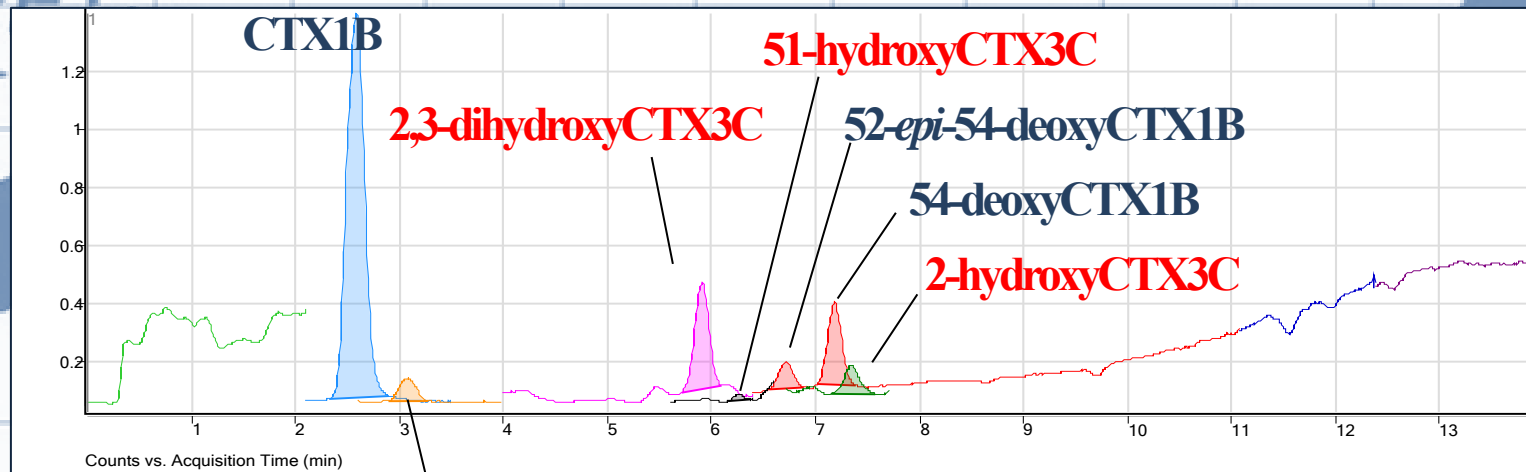
Toxin profile of *L. bohar* from Minamitorishima Island

Japan
Minamitorishima Isl.
Okinawa
2700 km

Hawaii

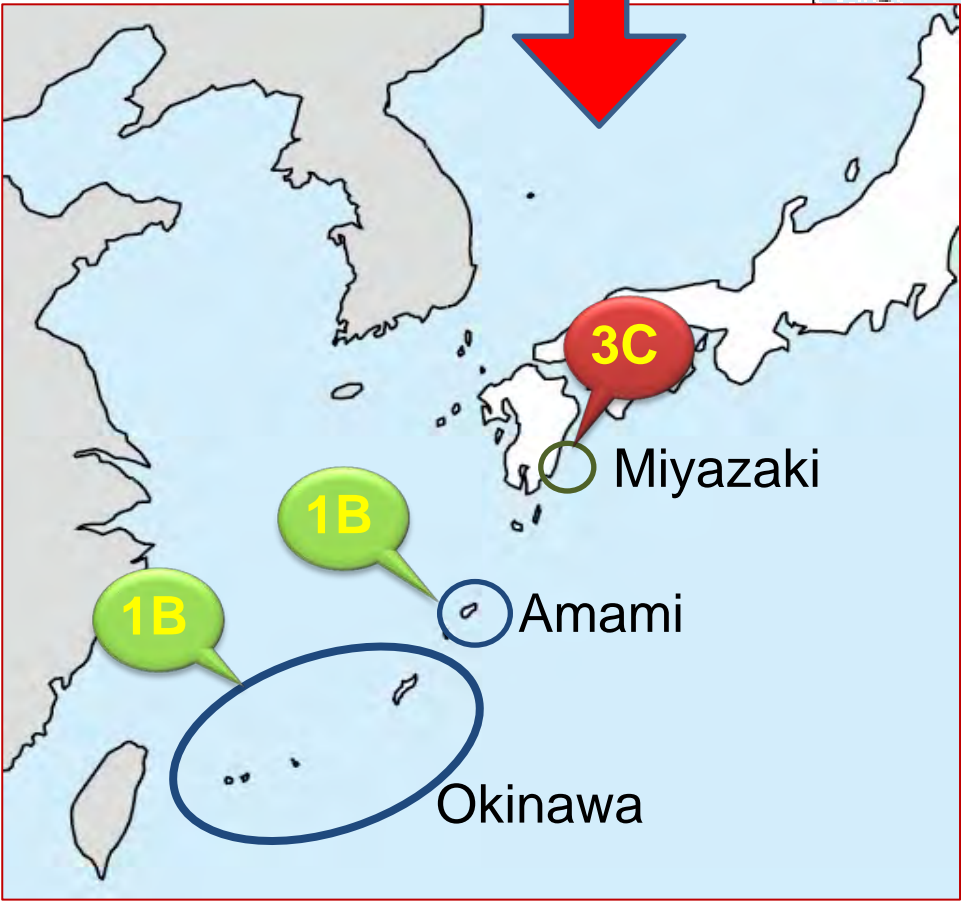
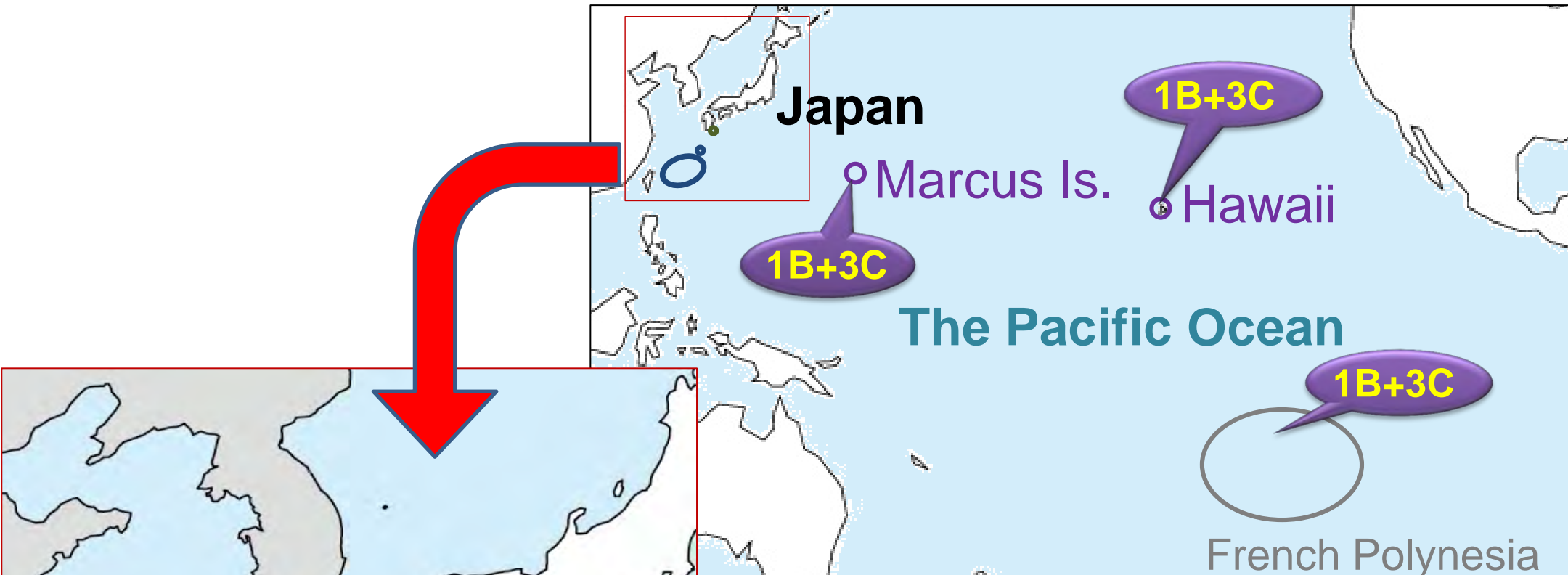


Lutjanus bohar



2,3,51-trihydroxyCTX3C : deduced from $[M+Na]^+$ and R_t

Both CTX1B and CTX3C congeners were detected



Regional distinction of Toxin Profile

Origin of Toxin producer is different?

Acknowledgement

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- Dr. Sato (Cell Science Institute co.)
- Dr. Hidemasa HIGA (Chibana Clinic)