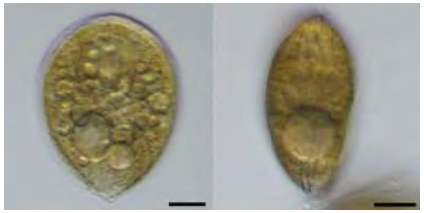
Discovery of novel ovatoxin isomers in several *Ostreopsis* strains in Japan



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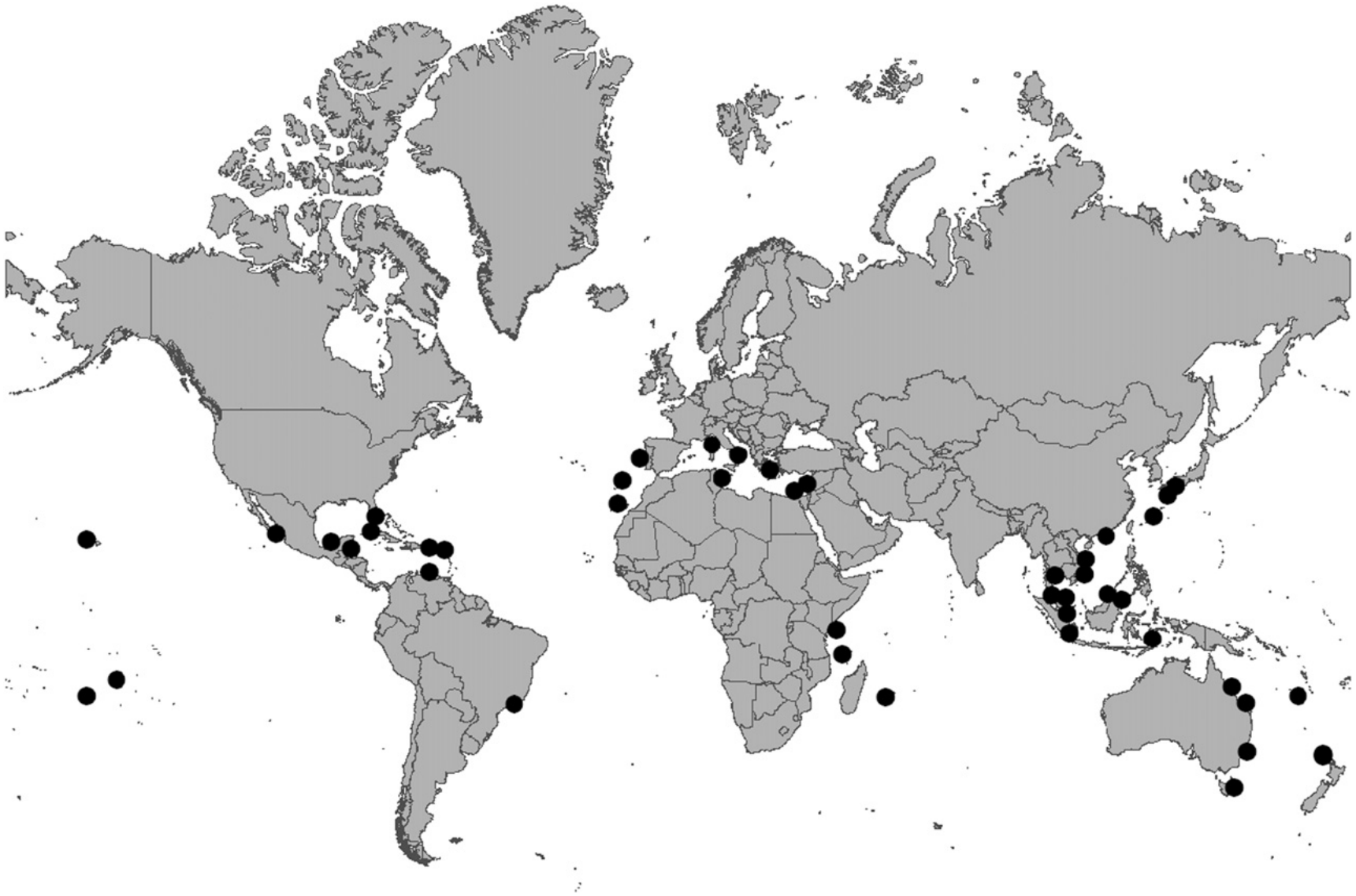
Ostreopsis spp.

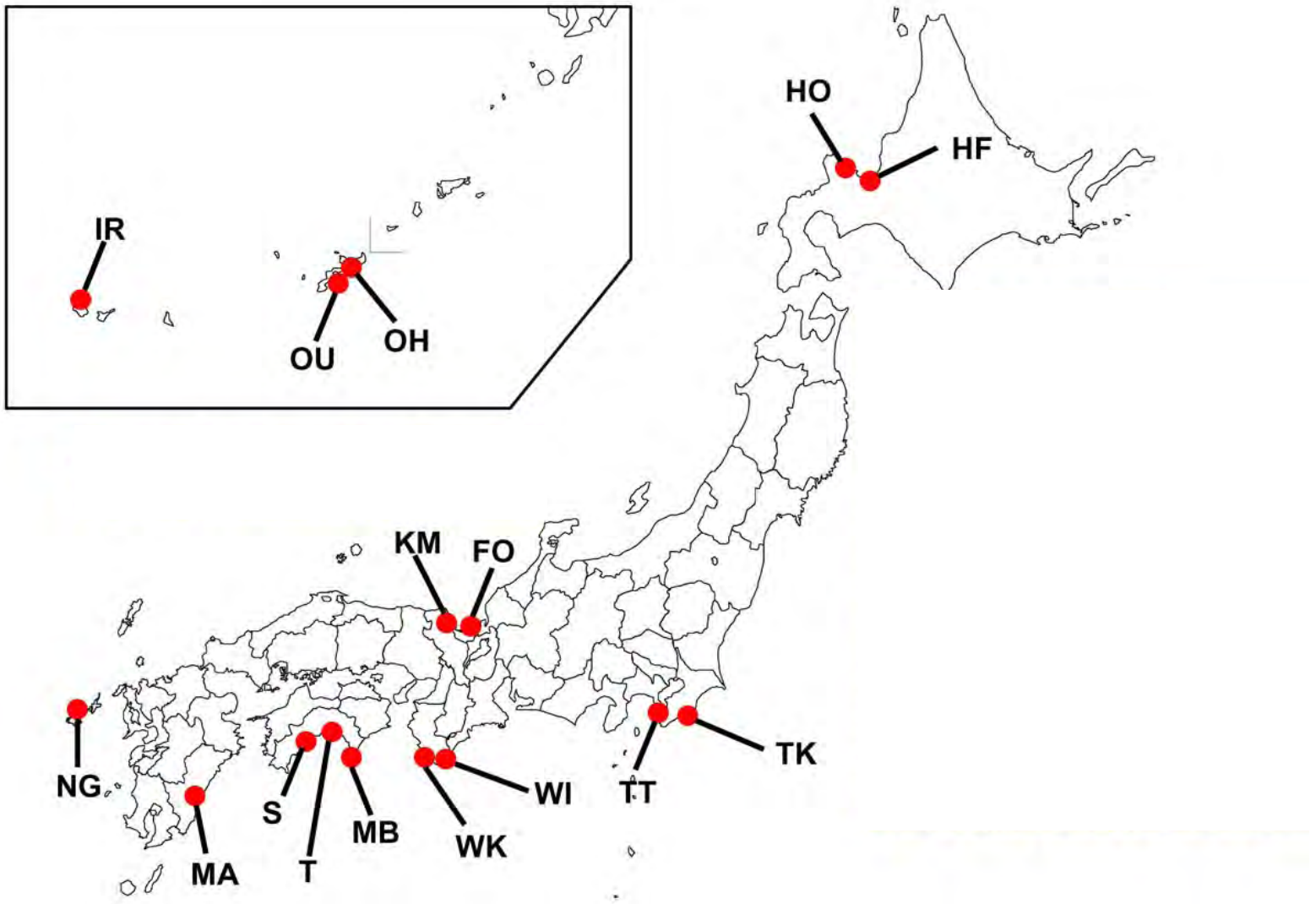
Dinoflagellate genus
Ostreopsis spp.
（*O.siamensis*　*O. ovata* etc 9 species）

- The dinoflagellate genus, *Ostreopsis*, has an increasingly global distribution
- Some *Ostreopsis* produces palytoxin analogues
- *O. ovata* is held responsible for respiratory illnesses due to inhalation of aerosols during blooms in the Mediterranean region



(Rhodes et al, Toxicon 2010)

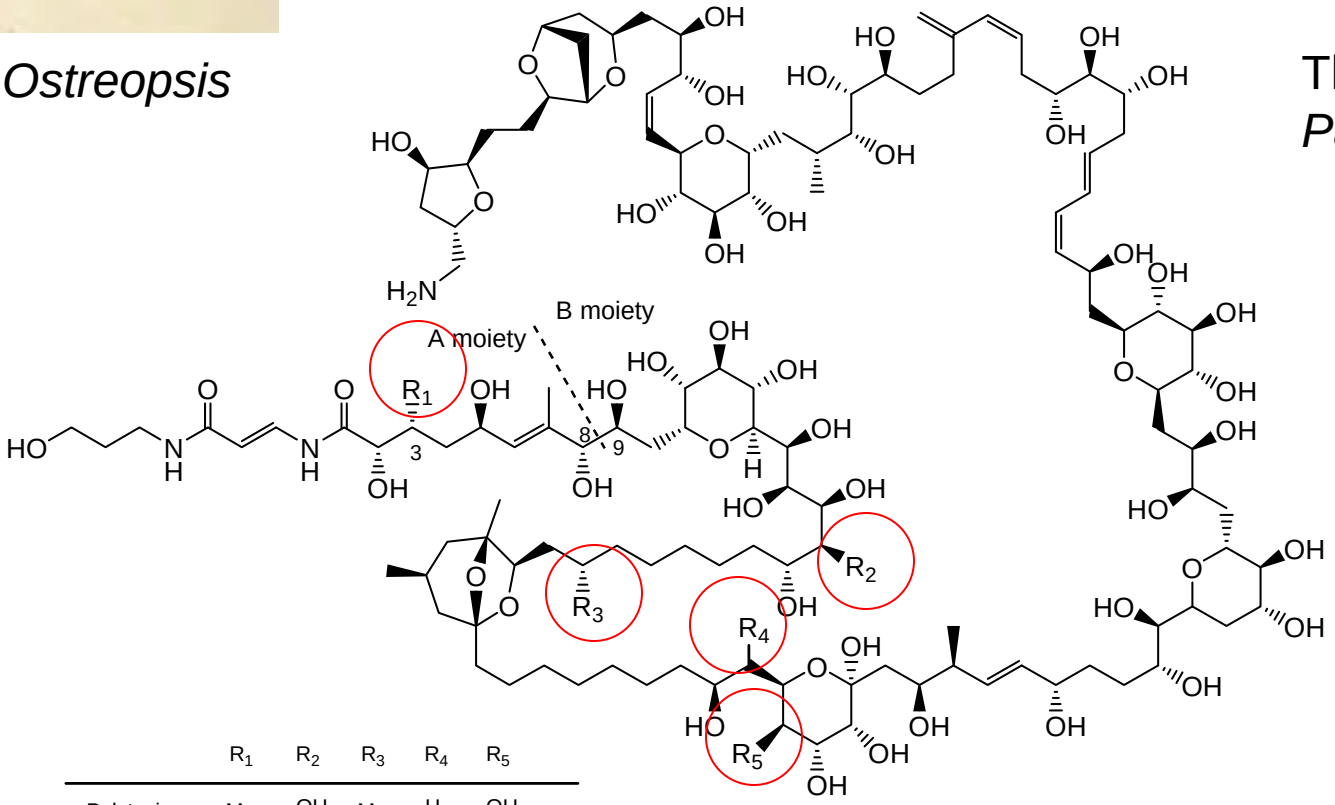
Ostreopsis found along the coastal area in Japan



Palytoxins

Ostreopsis

The Soft coral
Palythoa toxica



	R_1	R_2	R_3	R_4	R_5
Palytoxin	Me	OH	Me	H	OH
Ostreocin-D	H	H	H	OH	H

Human poisoning cases due to consumption of seafood suspected to be contaminated with palytoxins

- Human fatalities due to consumption of seafood suspected to be contaminated with palytoxins were reported in the Philippines, after consumption of the crab *Demania reynaudii* (1988), and in Madagascar following consumption of the sardine *Herklotsichthys quadrimaculatus* (clupeotoxism) (1999)

- Respiratory illness has also occurred when people were exposed to *Ostreopsis ovata* bloom aerosols during recreational or working activities, in Italy (2006)

D. reynaudii

H. quadrimaculatus

Ostreopsis ovata

Blue humphead parrotfish (*Scarus ovifrons*) poisoning in Japan (Palytoxin like poisoning)

- Symptoms: Rhabdomyolysis, a syndrome injuring skeletal muscle, causing muscle breakdown, and leakage of large quantities of intracellular (myocyte) contents into blood plasma
- The symptoms are similar to palytoxin poisoning
- Palytoxins have not been confirmed yet from the blue humphed parrotfish which was identified as the causative food in the poisoning

Ostreopsis

?

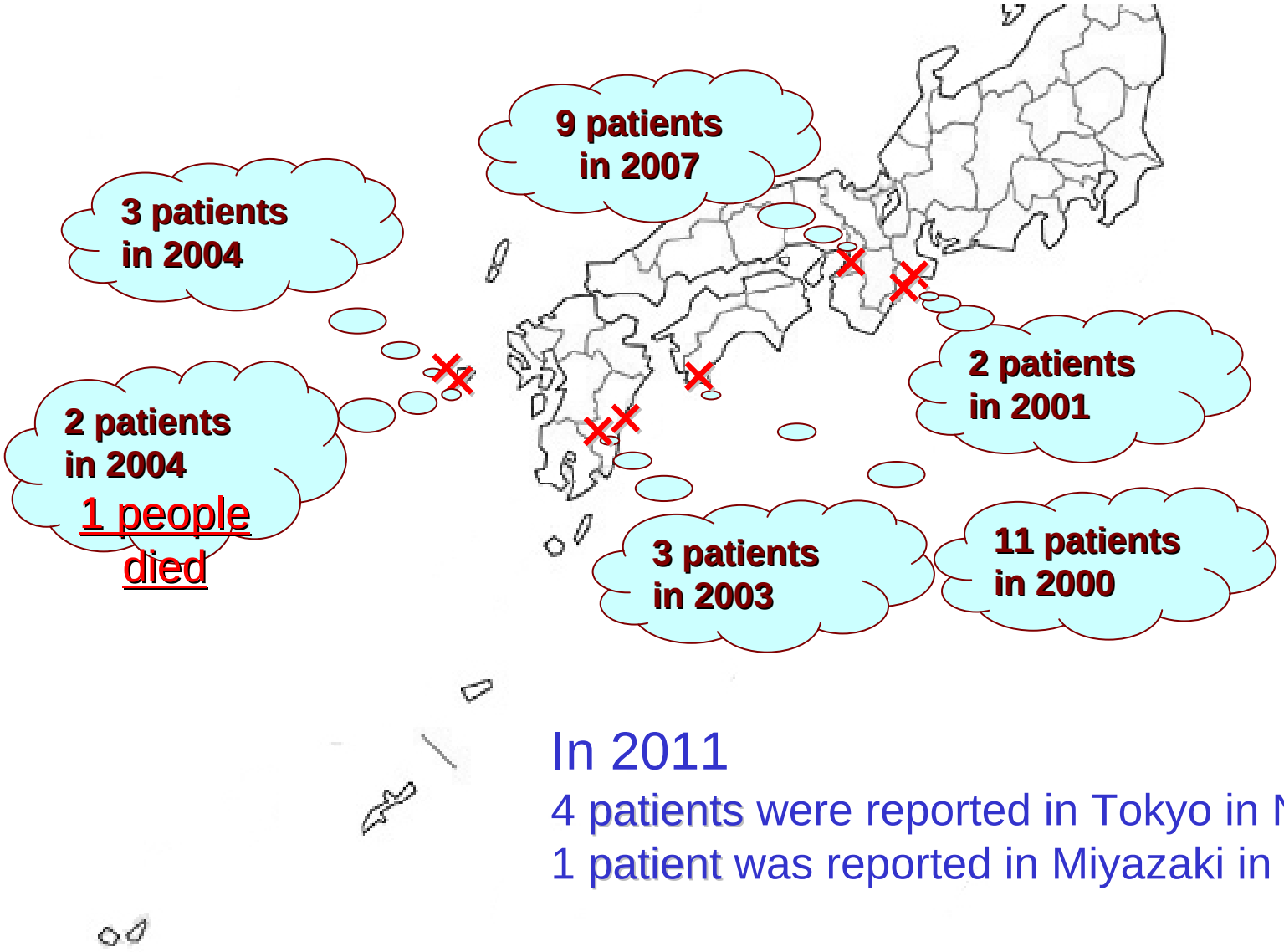
palytoxin

The Soft coral *Palythoa* spp.

?

Scarus ovifrons

Blue humphead parrotfish (*Scarus ovifrons*) poisoning in Japan (Palytoxin like poisoning)



In 2011

4 patients were reported in Tokyo in November

1 patient was reported in Miyazaki in March

Objectives

- LC-MS/MS analysis of palytoxin analogues in several *Ostreopsis* strains collected in Japan

- LC-MS/MS analysis of palytoxin analogues in the blue humphead parrotfish (*Scarus ovifrons*) which was identified as the causative food in human poisoning cases in Tokyo and Miyazaki in 2011

LC-MS/MS analysis of palytoxin analogues in several *Ostreopsis* strains collected in Japan

References

- Ciminiello, P. et al. 2006. The Genoa 2005 outbreak. Determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. Anal. Chem. 78, 6153–6159.
- Ciminiello, P. et al. 2008. Putative palytoxin and its new analogue, ovatoxin-a, in *Ostreopsis ovata* collected along the Ligurian coasts during the 2006 toxic outbreak. J. Am. Soc. Mass Spectrom. 19, 111–120.
- Ciminiello, P. et al. 2010. Complex palytoxin-like profile of *Ostreopsis ovata*. Identification of four new ovatoxins by high-resolution liquid chromatography/mass spectrometry. Rapid commun. Mass Spectrom. 24, 2735–2744.

LC-MS/MS chromatogram of palytoxin

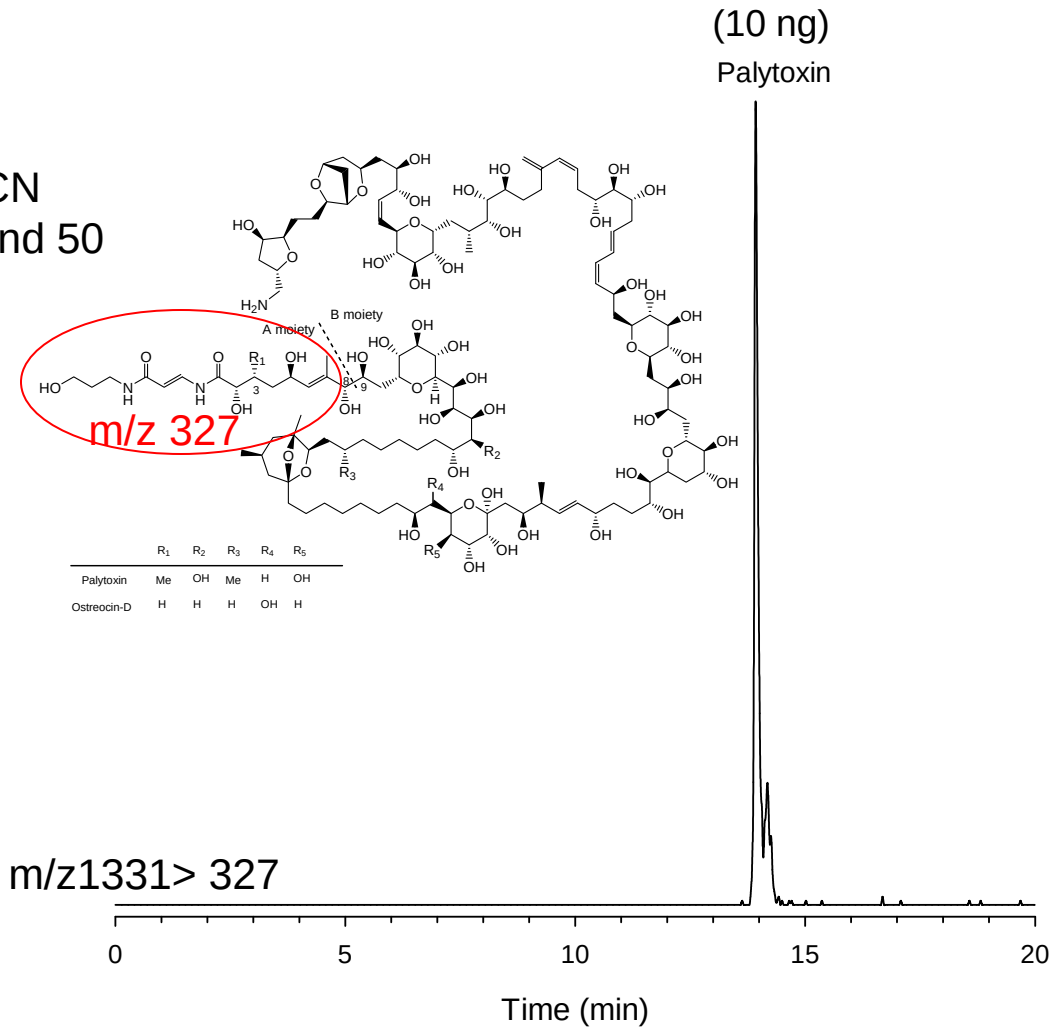
LC-MS condition

Column: Hypersil-BDS-C8
(150 mm x 2 mm i.d)
Flow rate: 0.2 mL/min
Mobile phase: A water, B 95% MeCN both containing 2 mM $HCOONH_4$ and 50 mM HCOOH

Step 1: 5 % B 100%B for 15 min
Step 2: 100 % B for 5 min

Detection
$[M+2H-H_2O]^{2-}$ 1331> 327

LOD：10ng/ml (0.1ng)



LC-MS/MS chromatogram of palytoxin analogues in *Ostreopsis* collected in coastal waters in Japan

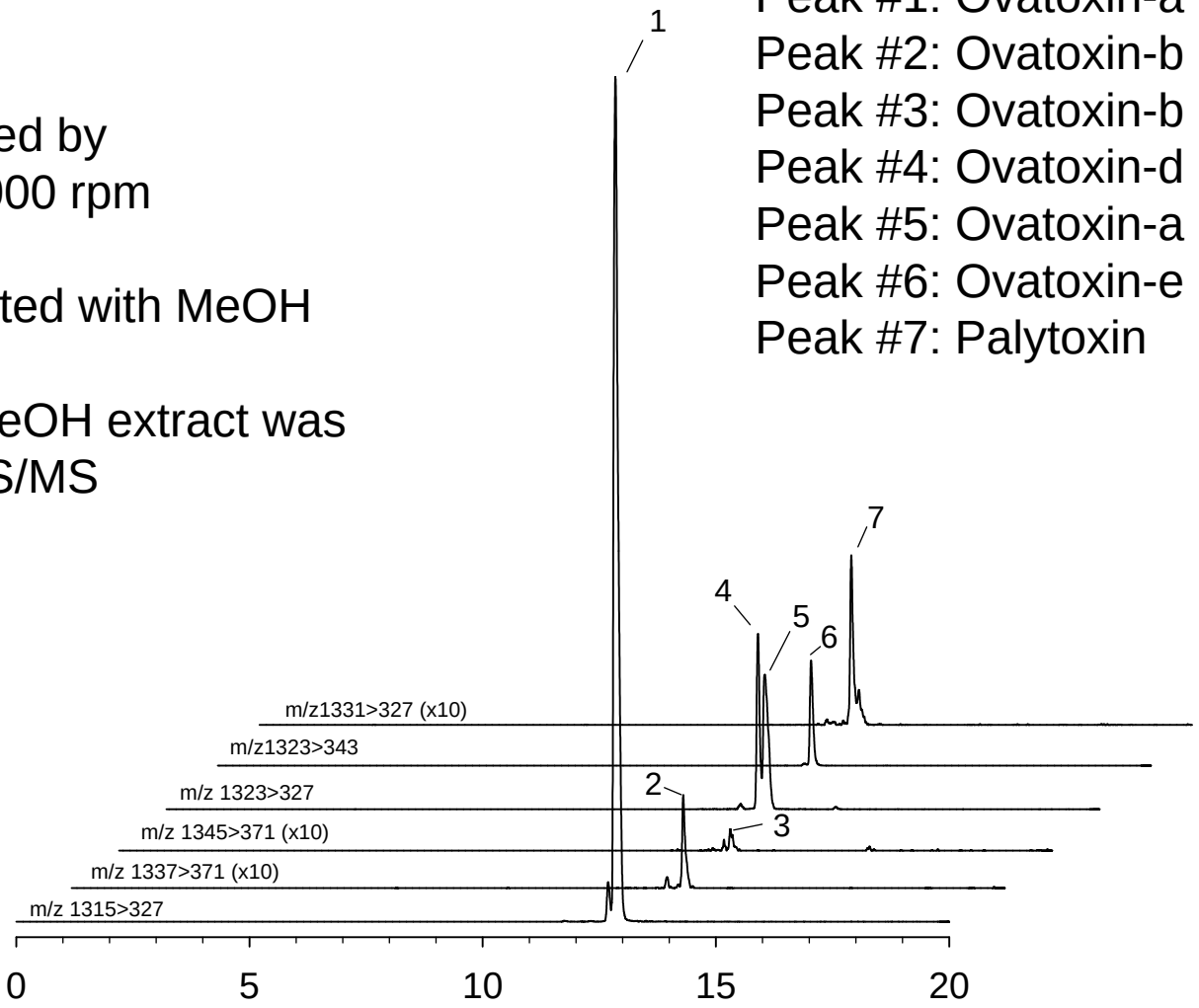
Ostreopsis culture

Cells were harvested by centrifugation at 3000 rpm

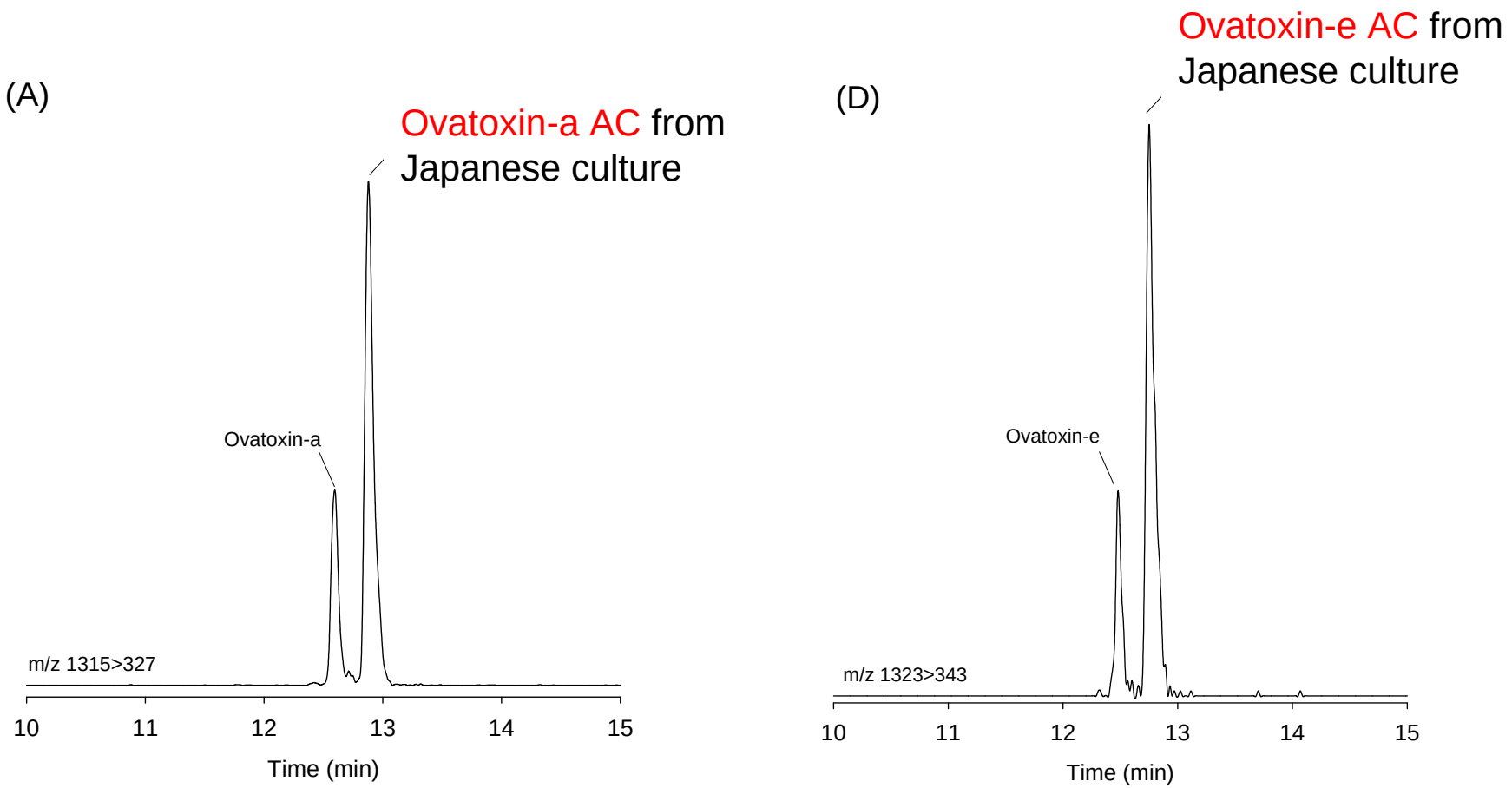
Toxins were extracted with MeOH

An aliquot of the MeOH extract was analyzed by LC-MS/MS

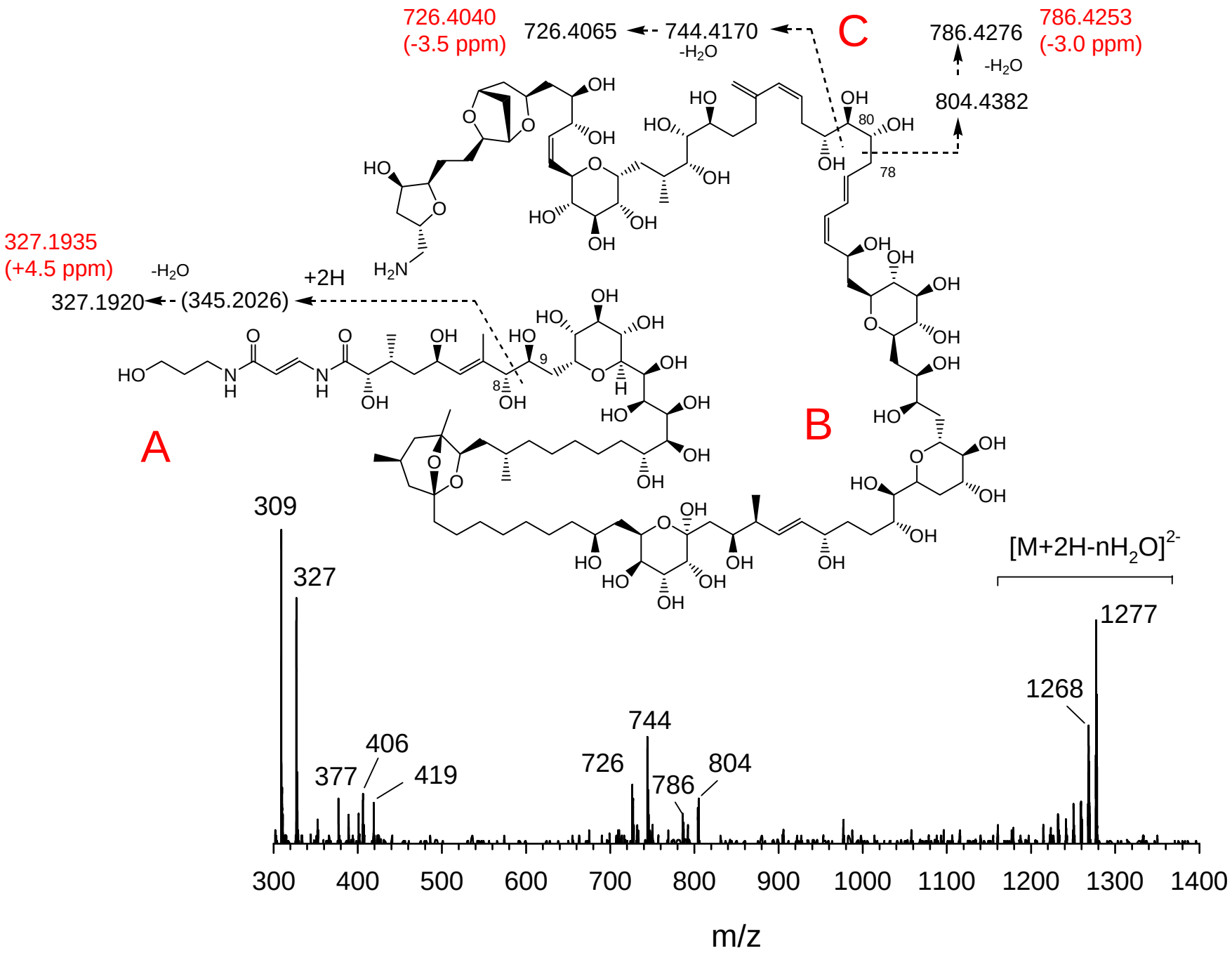
Peak #1: Ovatoxin-a
Peak #2: Ovatoxin-b
Peak #3: Ovatoxin-b isotope
Peak #4: Ovatoxin-d
Peak #5: Ovatoxin-a isotope
Peak #6: Ovatoxin-e
Peak #7: Palytoxin



LC–MS/MS chromatogram obtained from the mixture of Italian and Japanese *Ostreopsis* strain extracts

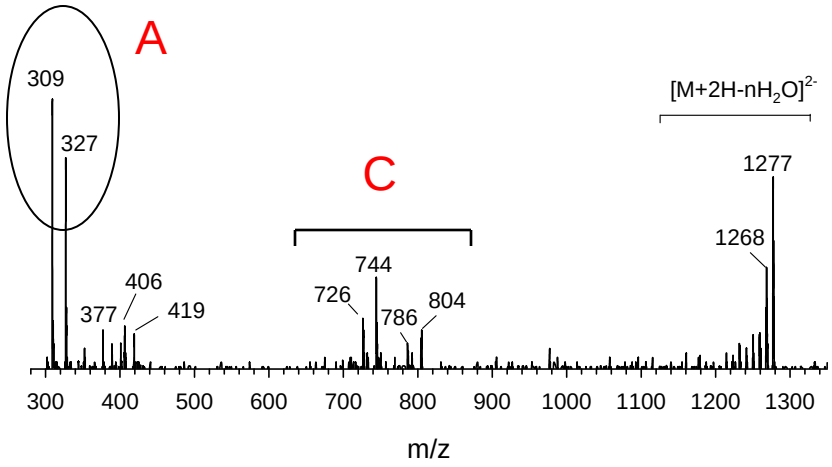


AC: Porf. Adachi Culture

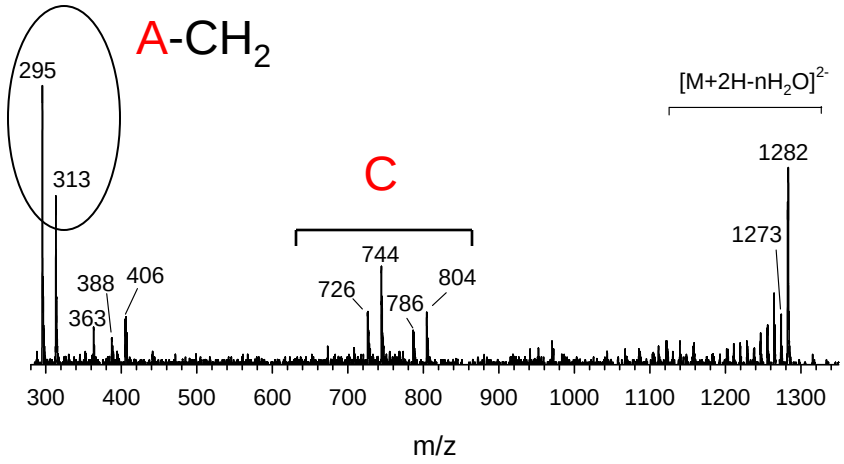

726.4040
(-3.5 ppm)
726.4065
744.4170
-H2O
C
786.4276
-H2O
786.4253
(-3.0 ppm)
804.4382
327.1935
(+4.5 ppm)
-H2O
+2H
327.1920
(345.2026)
A
B
309
327
406
377
419
744
726
786
804
1277
1268
[M+2H-nH2O]2-
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

LC–MS/MS spectra of palytoxin analogues in *Ostreopsis*

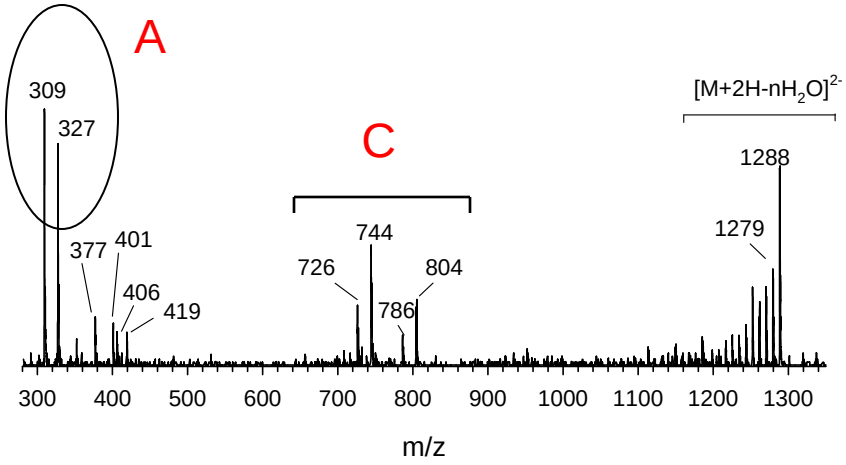
Palytoxin



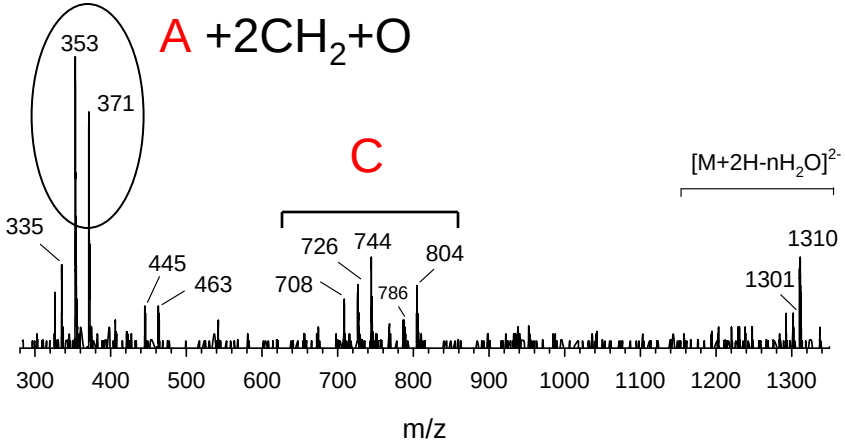
Ostreocin-D



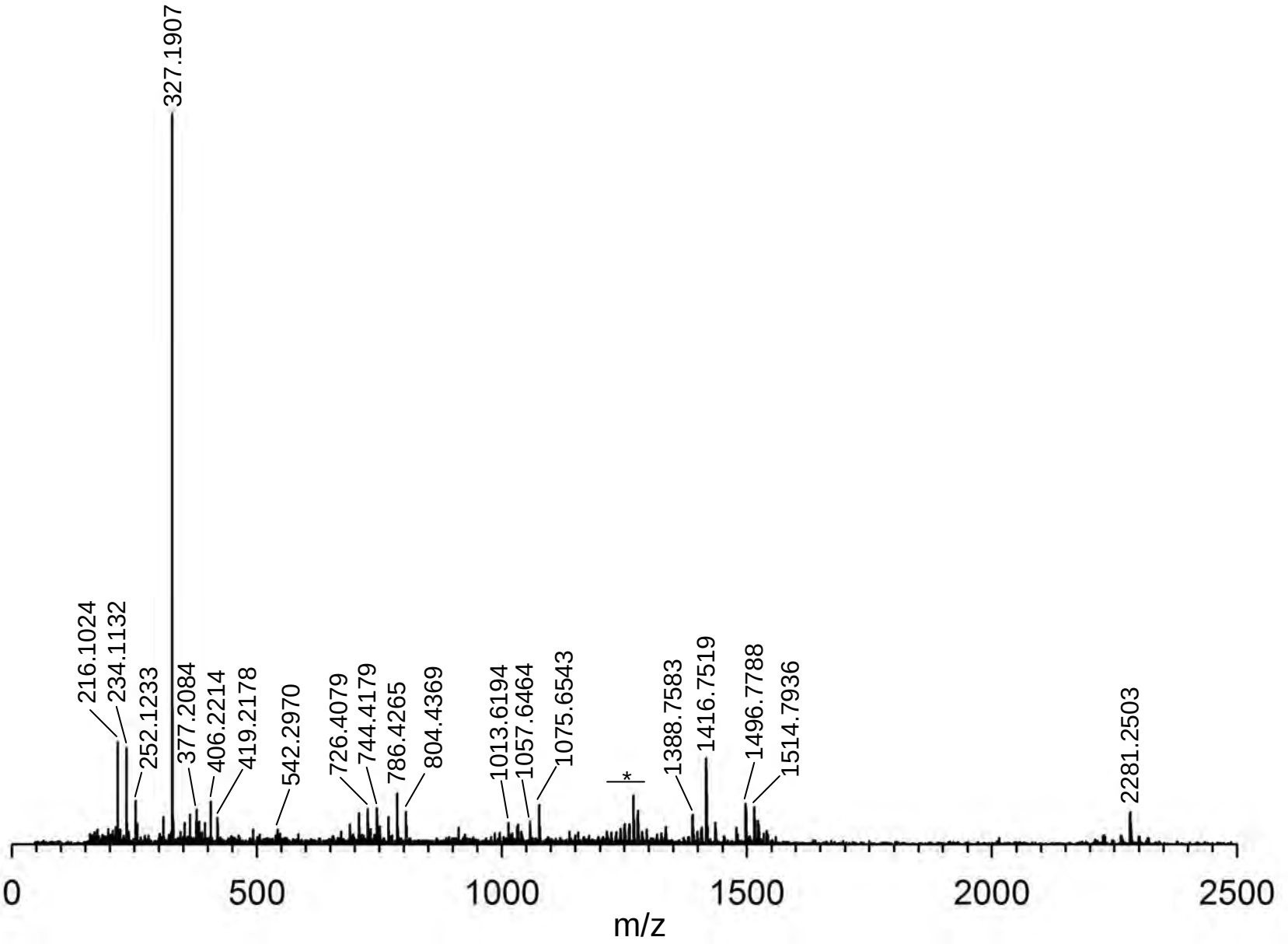
Ovatoxin-a AC



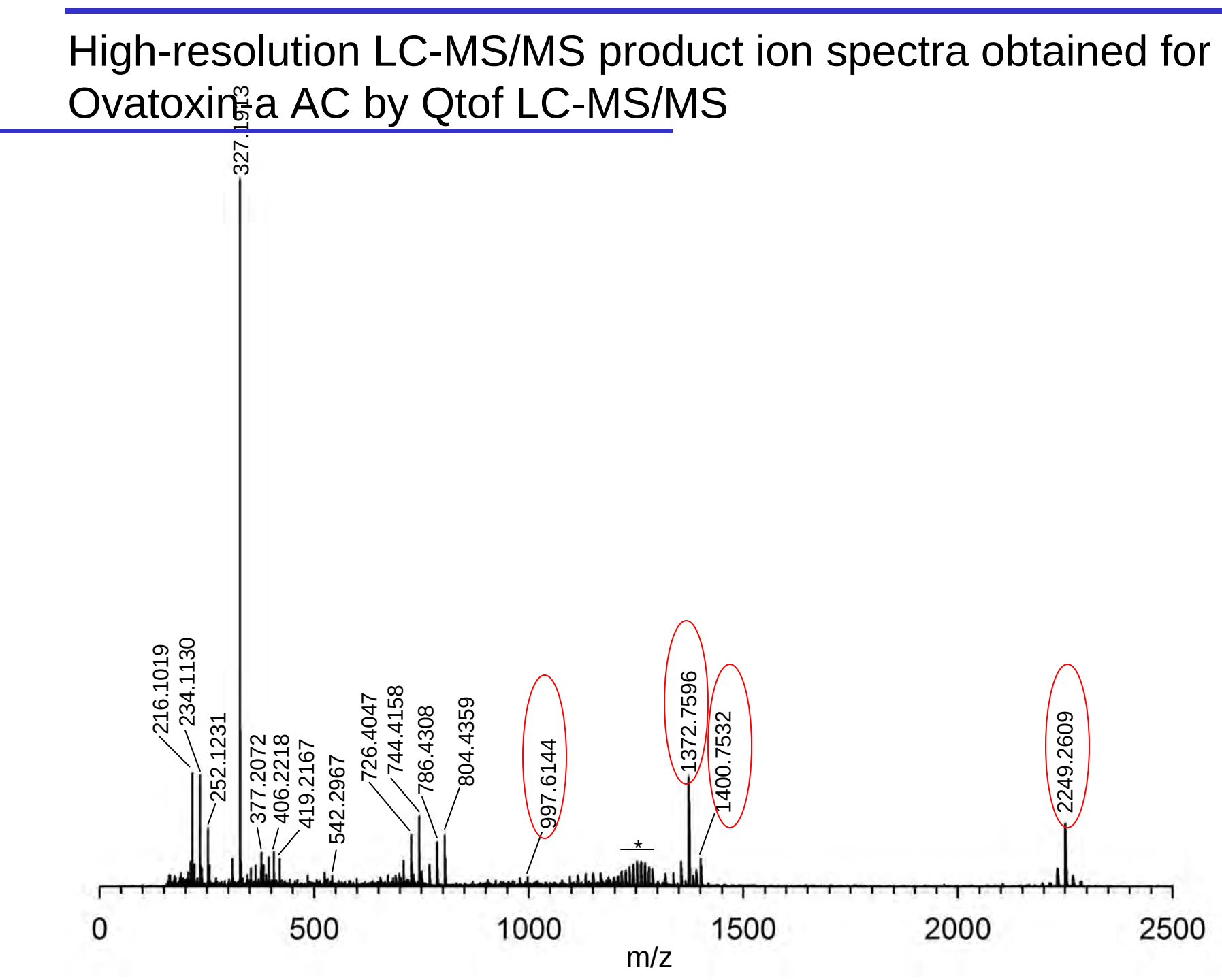
Ovatoxin-b



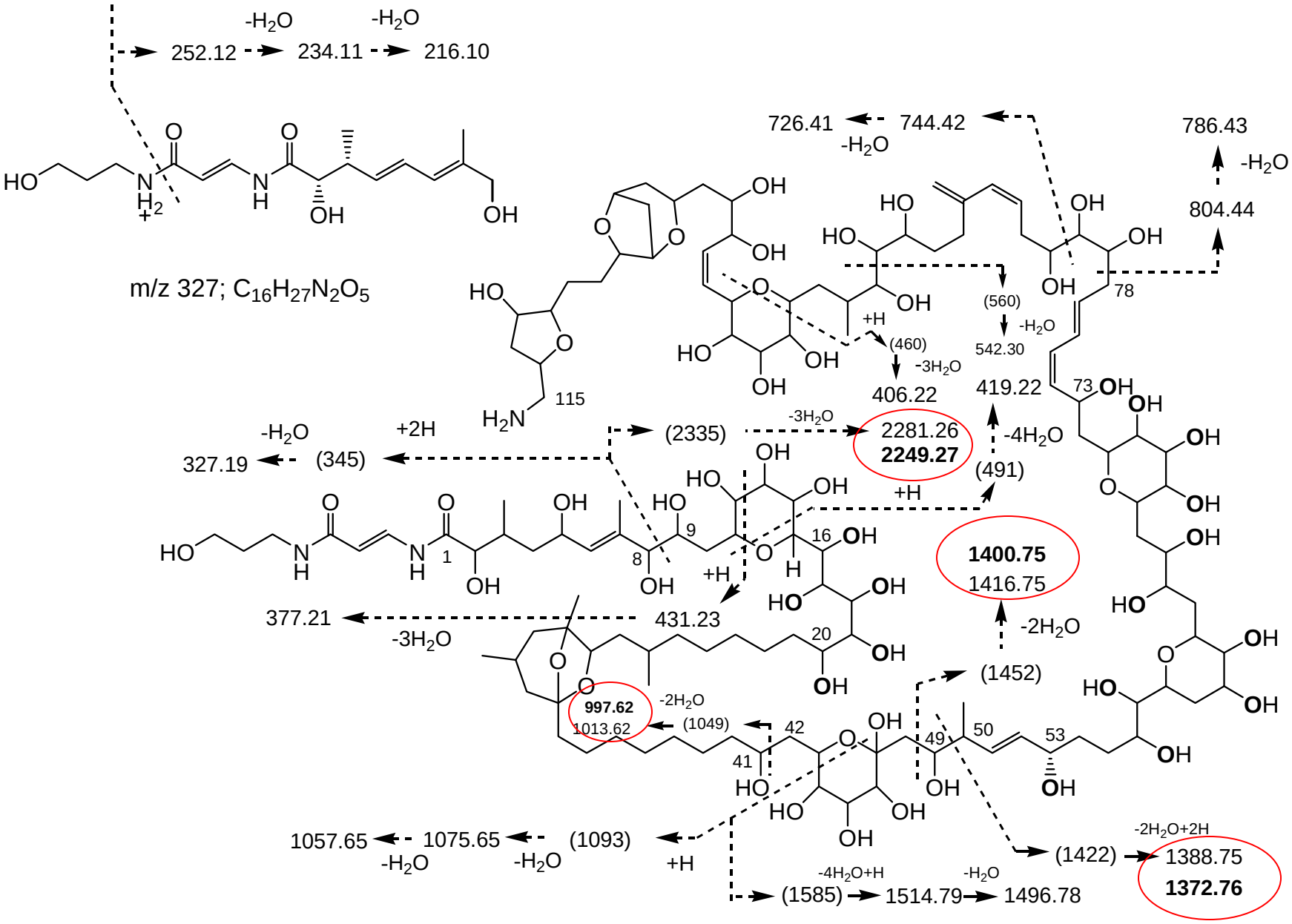
High-resolution LC-MS/MS product ion spectra obtained for authentic palytoxin standard by Qtof LC-MS/MS



High-resolution LC-MS/MS product ion spectra obtained for Ovatoxin-a AC by Qtof LC-MS/MS



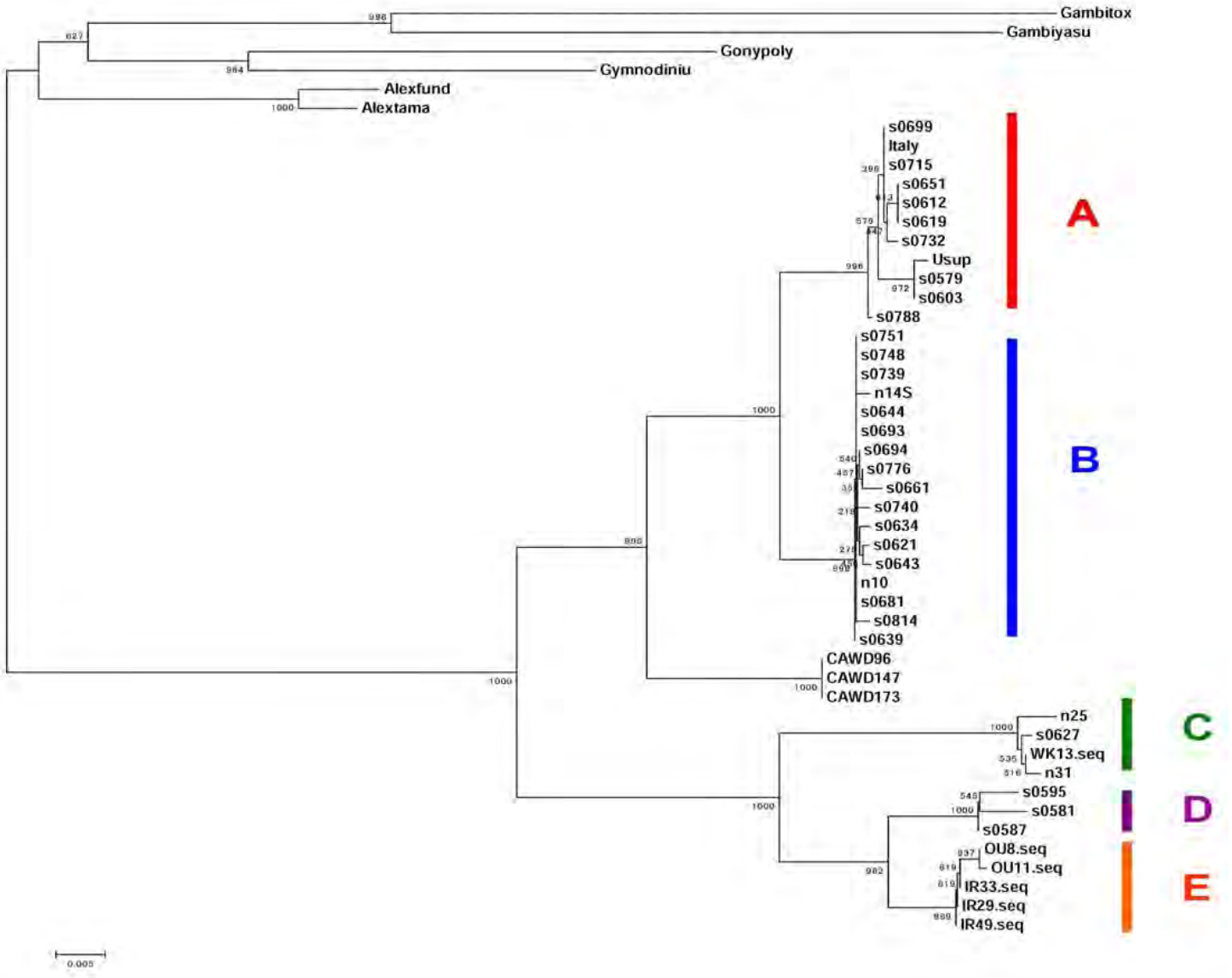
Proposed fragmentation diagram of palytoxin



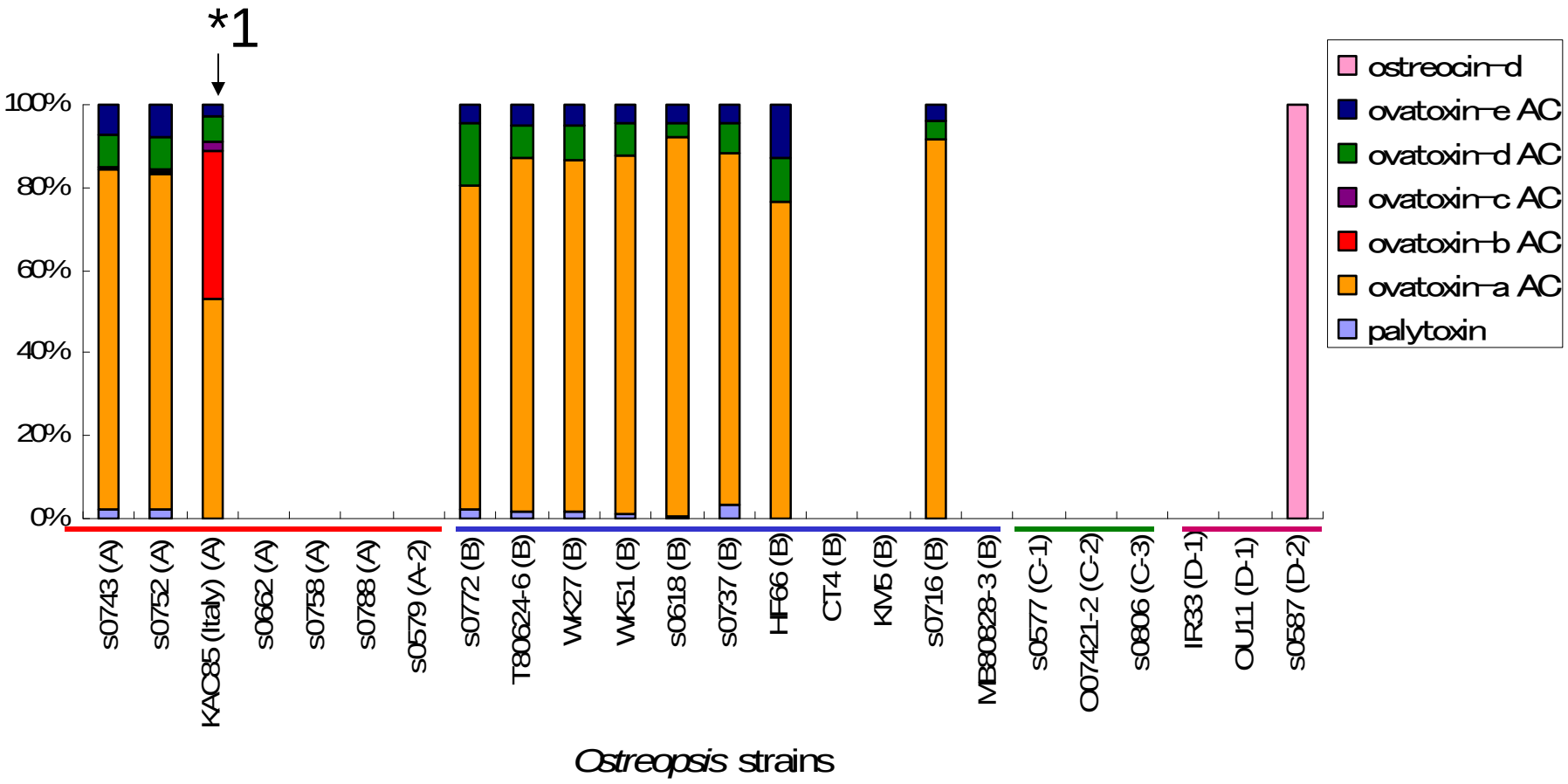
Elemental formulae of palytoxin analogues determined by QTOF LC-MS spectra on the positive mode

	m/z (measured value)	Formula	Tolerance (ppm)
Ovatoxin-a AC	2647.5062	$C_{129}H_{224}N_3O_{52}$	3.2
Ovatoxin-d AC	2663.4918	$C_{129}H_{224}N_3O_{53}$	-0.3
Ovatoxin-e AC	2663.4841	$C_{129}H_{224}N_3O_{53}$	-3.2

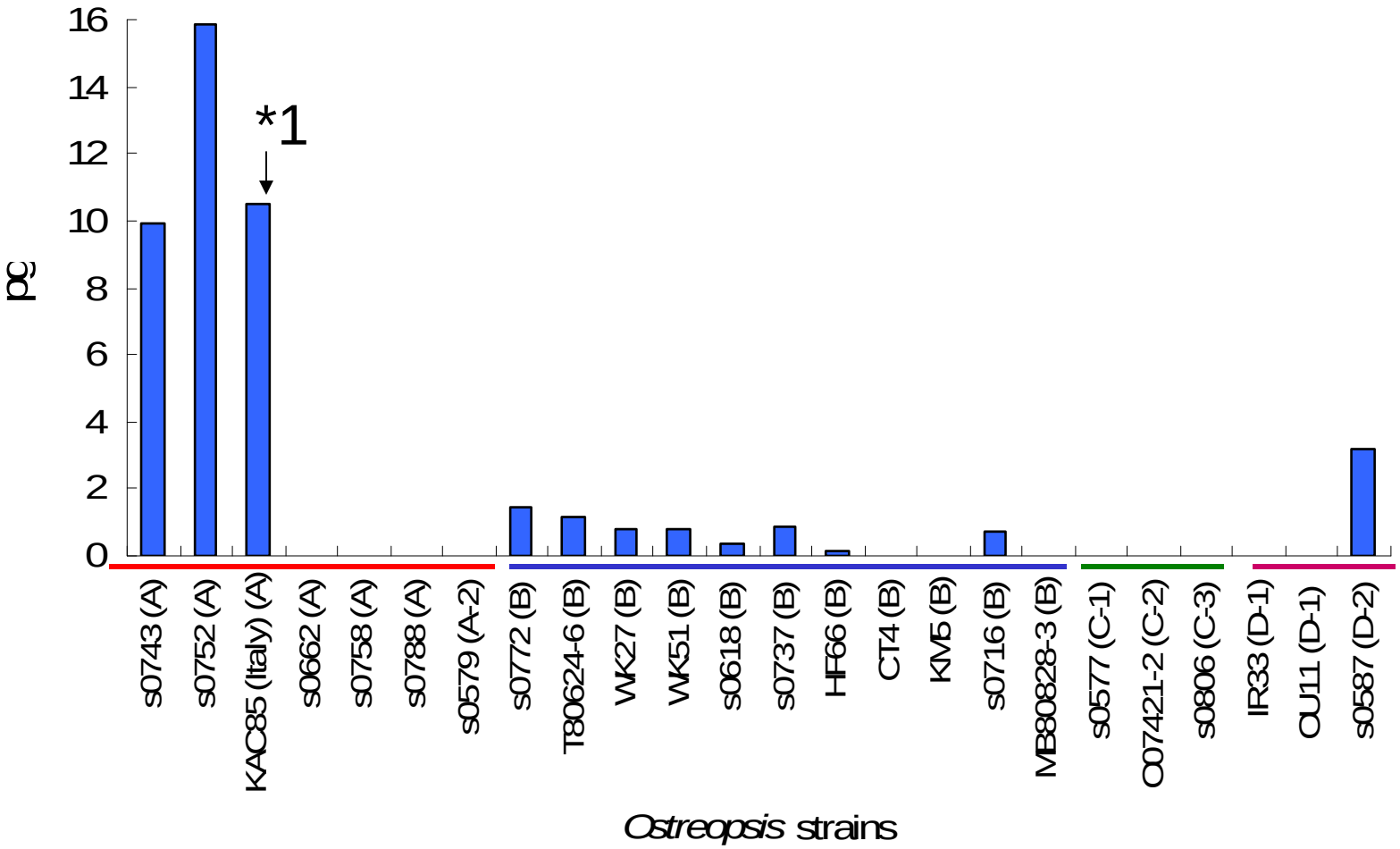
Clades classified by the phylogenetic analysis reported in our previous study (Sato et al., 2011)



Toxin profiles of several *Ostreopsis* strains collected in Japan and Italy (*1) analyzed by MRM LC-MS/MS



Total cellular toxin contents of several *Ostreopsis* strains collected in Japan and Italy (*1) analyzed by MRM LC-MS/MS



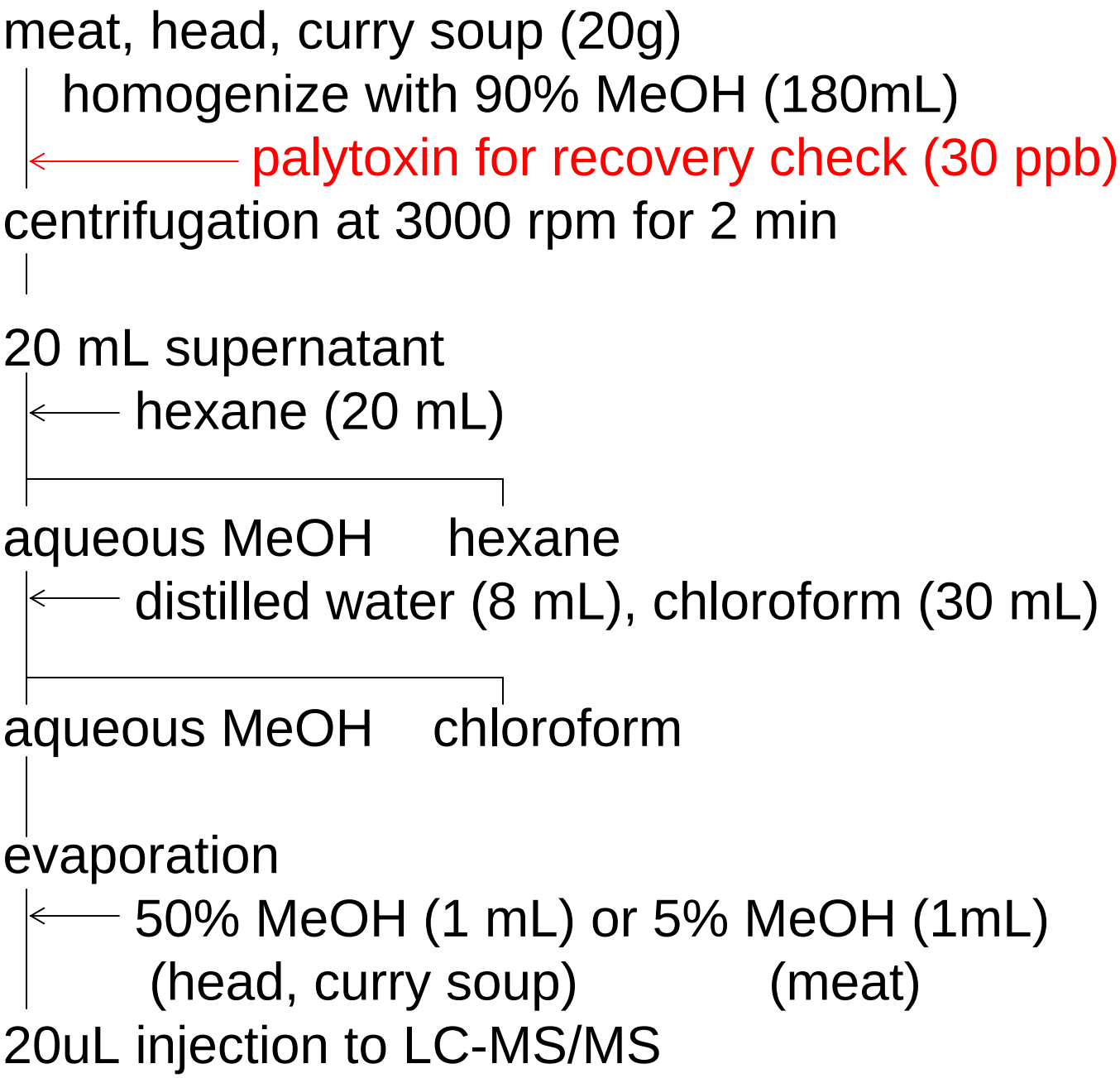
Summary

- Novel isomers of ovatoxin-a, -b, -d, -e were found in Japanese *Ostreopsis*. The isomers of ovatoxin-a,-b,-d, and –e detected in Japanese *Ostreopsis* were tentatively named ovatoxin-a AC, -b AC,-d AC and –e AC.
- LC-MS analysis revealed that ovatoxin-a AC is a structure analogue of palytoxin reduced a hydroxyl group at both C16-C20 and C53-C73 moieties of palytoxin.
- Toxin profiles of *Ostreopsis* collected in middle to southern coastal area in Japan were clarified by LC-MS/MS
- The toxin contents of some strains collected in Japan were comparable to that obtained in a strain (KAC 85) collected in the Italian coast where human health problems occurred

LC-MS/MS analysis of palytoxin analogues in the blue humphead parrotfish (*Scarus ovifrons*) which was identified as the causative food in human poisoning cases in Tokyo and Miyazaki in 2011

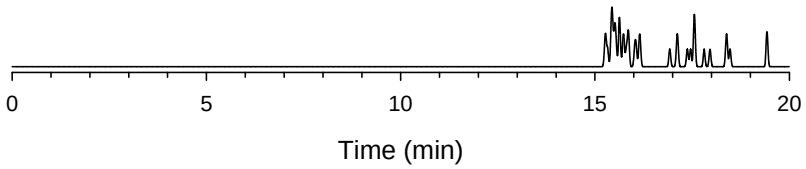
Human poisoning cases by consumption of the blue humphead parrotfish in 2011

- In March 2011, the human poisoning case by consumption of the blue humphead parrotfish occurred in Miyazaki. One patient was diagnosed as the palytoxin like poisoning
- In November 2011, the human poisoning case by consumption of the blue humphead parrotfish occurred in Tokyo. Four patients were diagnosed as the palytoxin like poisoning
- The blue humphead parrotfish samples caused the human poisoning were provided to NRIFS to identify palytoxin by LC-MS/MS

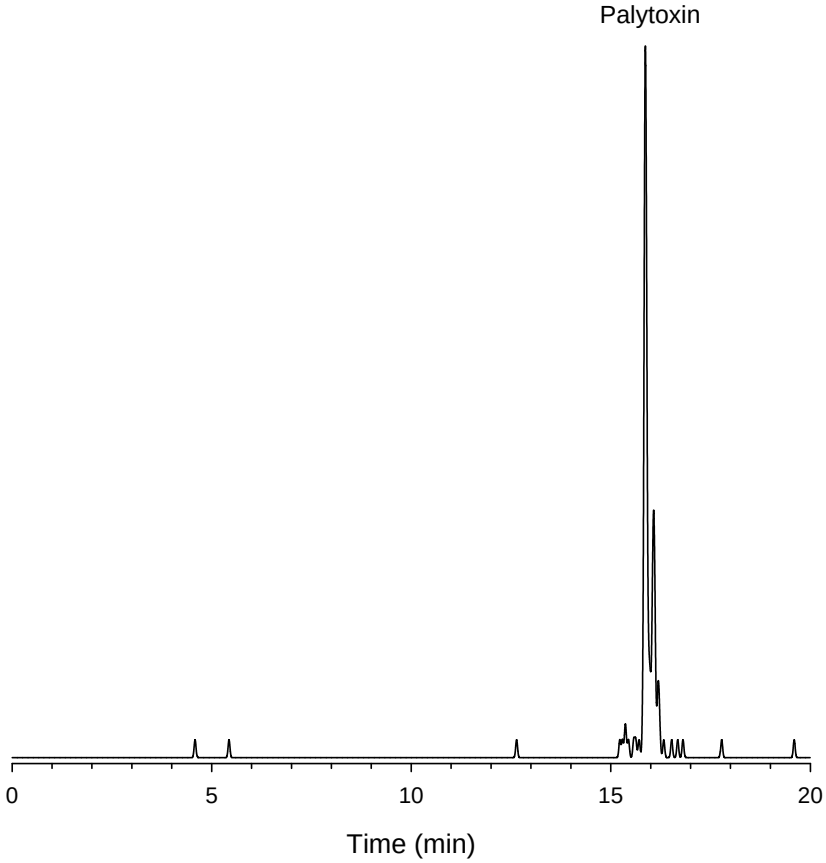

meat, head, curry soup (20g)
homogenize with 90% MeOH (180mL)
palytoxin for recovery check (30 ppb)
centrifugation at 3000 rpm for 2 min
20 mL supernatant
hexane (20 mL)
aqueous MeOH
hexane
distilled water (8 mL), chloroform (30 mL)
aqueous MeOH
chloroform
evaporation
50% MeOH (1 mL) or 5% MeOH (1mL)
(head, curry soup)
(meat)
20uL injection to LC-MS/MS

LC-MS/MS analysis of the blue humphead parrotfish

Fish meat causing of
the human poisoning case (A)



Fish meat (A) fortified with
palytoxin at 30 ppb



Recovery (%) of palytoxin from the blue humphead parrotfish fortified with palytoxin at 30 ppb

	Recovery (%)	LOQ (ppb)	LOD (ppb)
Head	58.3	1.18	0.35
Curry soup	66.7	1.50	0.45
Meat	57.1	1.60	0.48

Summary

- LC-MS/MS method of palytoxin analogues in cooked seafood samples was developed
- The LOD and LOQ are 0.5 and 2 ppb those are lower than the EFSA regulatory level (30 ppb) of palytoxins in seafood
- Palytoxins were not detected in the blue humphead parrotfish samples causing of the human poisoning in 2011
- Palytoxins are not the causative toxin in the blue humphead parrotfish poisoning in Japan