The Amazing Structural Variability of Phycotoxins and Implications for Monitoring and Regulation

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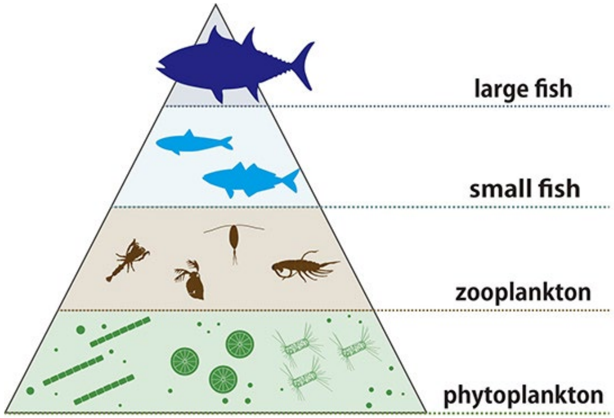
Introduction

- Marine toxins = Phycotoxins are toxic chemicals produced by photosynthetic plankton-species
- Dinoflagellates are the principle producers of phycotoxins
  →Also toxigenic diatoms or cyanobacteria amongst others
- Accumulate in a variety of filter feeding bivalves or shellfish and can reach high concentrations during algal blooms
  → "Harmful algal blooms" = HABs

Algal Bloom Lake Erie



Azaspiracids (AZA)

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1995: 8 people in the Netherlands became ill after consumption of Irish mussels (*Mytilus edulis*) harvested at Killary Harbour (Ireland). Symptoms were like DSP intoxication, but DSP toxins were hardly present in the mussels
(Harmful Algae News, 14, 2)

1998: Satake et al. identified azapiracid-1 (AZA-1) as the causative compound in shellfish (J. Am. Chem. Soc., 120, 9967-9968)

1999: Ofuji et al. elucidated the structures of AZA-2 and AZA-3 from shellfish
(Nat. Toxins, 7, 99-102)

2004: EFSA set a regulatory limit of 160 µg/kg fresh weight as the sum of AZA-1, -2, and -3.
(Off. J. Europ. Commun. L139/55ff.)

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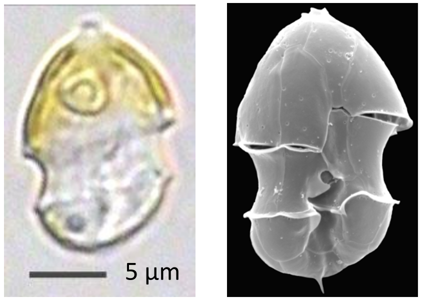
Azaspiracids (AZA)

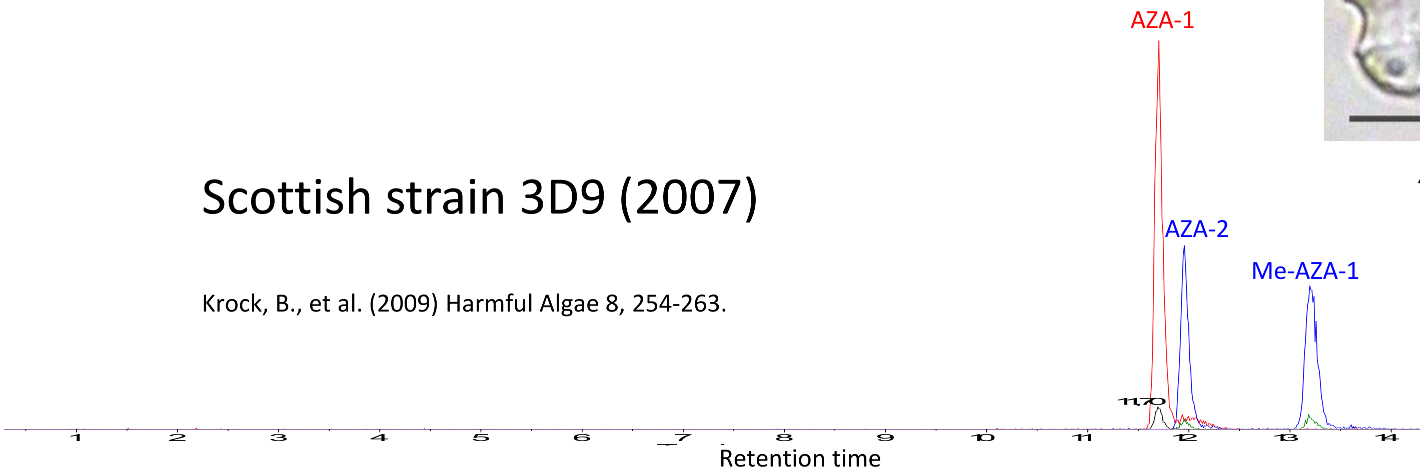
Azadinium as AZA producer



A. spinosum

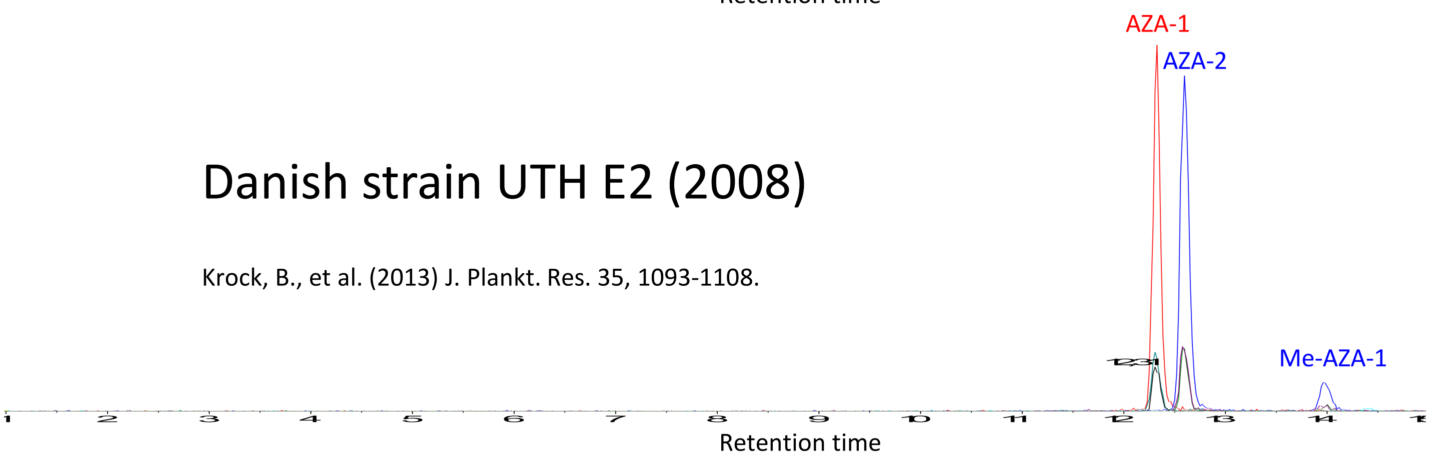
Scottish strain 3D9 (2007)

Krock, B., et al. (2009) Harmful Algae 8, 254-263.



Danish strain UTH E2 (2008)

Krock, B., et al. (2013) J. Plankt. Res. 35, 1093-1108.



m/z 842>824
m/z 856>838

Azaspiracids (AZA)

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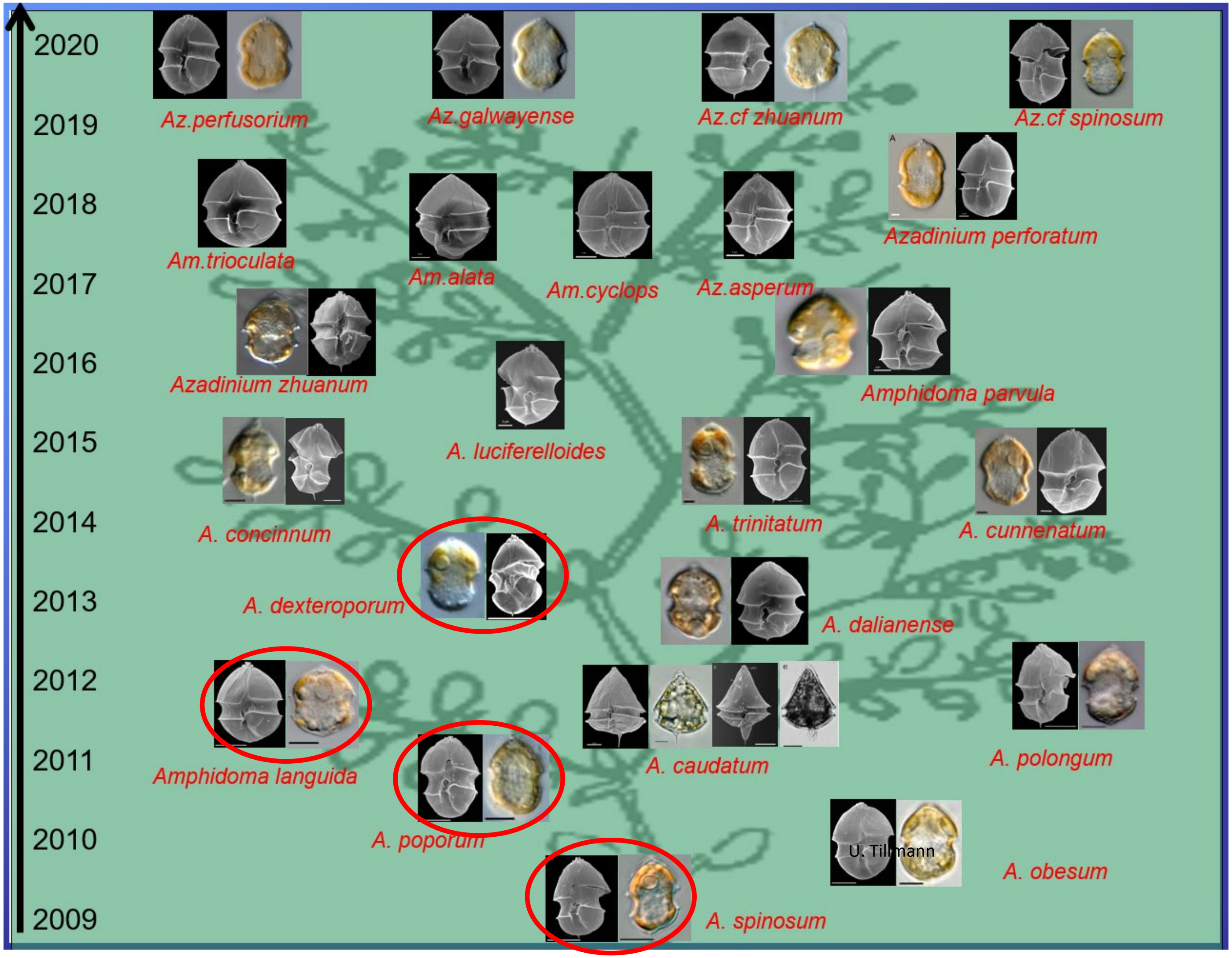
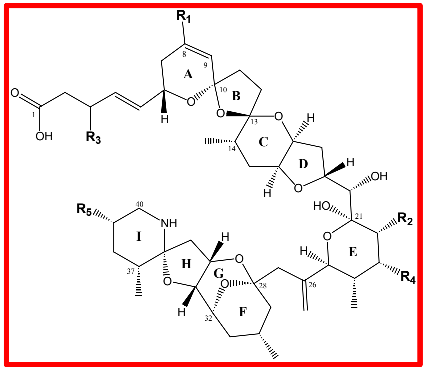
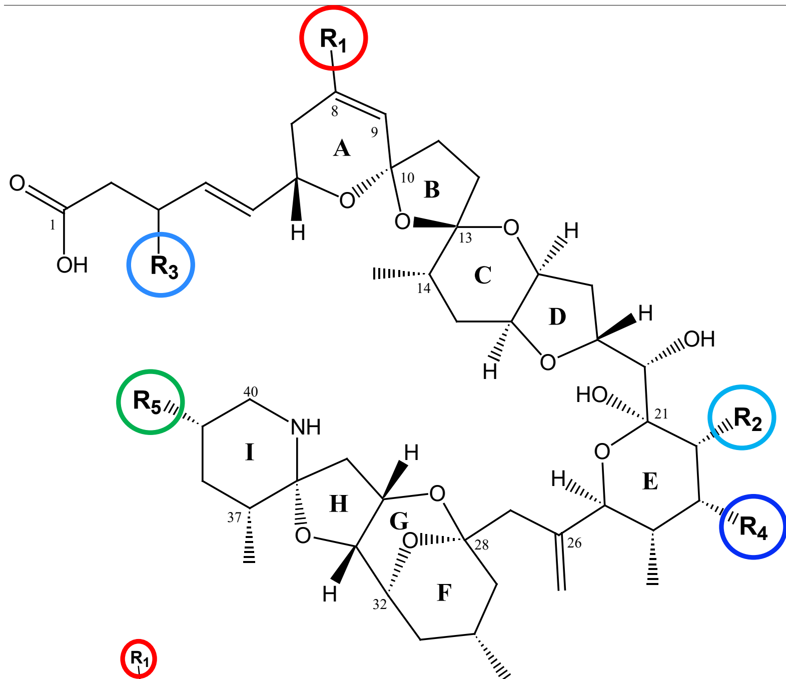
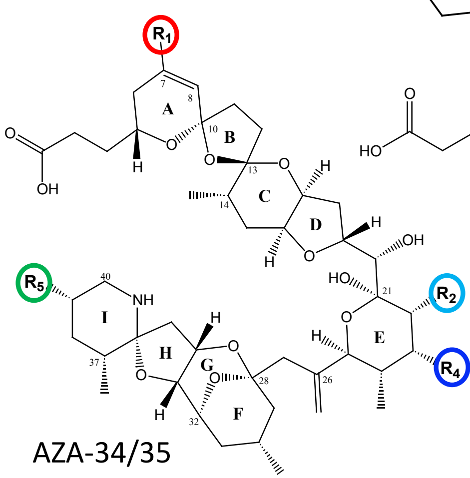


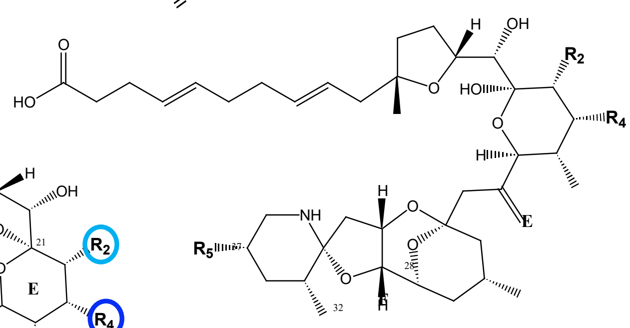


Azaspiracids (AZA)





AZA-34/35



AZA-33

Toxin	R_1	R_2	R_3	R_4	R_5	$\Delta_{7,8}$	$[M+H]^+$
AZA-1	H	CH_3	H	H	CH_3	√	842
AZA-2	CH_3	CH_3	H	H	CH_3	√	856
AZA-3	H	H	H	H	CH_3	√	828
AZA-4	H	H	OH	H	CH_3	√	844
AZA-5	H	H	H	OH	CH_3	√	844
AZA-6	CH_3	H	H	H	CH_3	√	842
AZA-7	H	CH_3	OH	H	CH_3	√	858
AZA-8	H	CH_3	H	OH	CH_3	√	858
AZA-9	CH_3	H	OH	H	CH_3	√	858
AZA-10	CH_3	H	H	OH	CH_3	√	858
AZA-11	CH_3	CH_3	OH	H	CH_3	√	872
AZA-33	-	CH_3	H	H	CH_3	-	716
AZA-34	H	CH_3	-	H	CH_3	√	816
AZA-35	CH_3	CH_3	-	H	CH_3	√	830
AZA-36	CH_3	CH_3	OH	H	H	√	858
AZA-37	H	CH_3	OH	H	H	-	846
AZA-38	nd	nd	nd	nd	H	nd	830
AZA-39	nd	nd	nd	nd	H	nd	816
AZA-40	CH_3	CH_3	H	H	H	√	842

Azaspiracids (AZA)

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AZA	R1	R2	R3	R4	R5	R6	R7	[M+H]+	Frag. Type	origin	status	reference
AZA1	H	H	H	CH3	H	CH3	CH3	842,5	362 · 262	A. spinosum	phycotoxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 · 262	spin/pop/lang	phycotoxin	Rehmann et al. 2008
AZA3	H	H	H	H	H	CH3	CH3	828,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA4	OH	H	H	H	H	CH3	CH3	844,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA5	H	H	H	H	OH	CH3	CH3	844,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA6	H	CH3	H	H	H	CH3	CH3	842,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA7	OH	H	H	CH3	H	CH3	CH3	858,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
epi AZA7	OH	H	H	CH3	H							Rossi et al. 2017
AZA8	H	H	H	CH3	OH							Rehmann et al. 2008
AZA9	OH	CH3	H	H	H							Rehmann et al. 2008
AZA10	H	CH3	H	H	OH							Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H						bol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH							Rehmann et al. 2008
AZA13	OH	H	H	H	OH							Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH							Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH							Rehmann et al. 2008
AZA16	OH	CH3	H	CH3	OH	CH3	CH3	888,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA17	H	H	H	COOH	H	CH3	CH3	872,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA18												Rehmann et al. 2008
AZA19	H	CH3	H	COOH	H	CH3	CH3	886,5	362 · 262	shellfish	metabolite	Rehmann et al. 2008
AZA20												Rehmann et al. 2008
AZA21	OH	H	H	COOH	H							Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H							Rehmann et al. 2008
AZA24												Rehmann et al. 2008
AZA25	H	H	H	H	H							Kilkoyne et al. 2018
AZA26	H	H	H	H	O							Kilkoyne et al. 2018
AZA27	H	CH3	H	H	H							Kilkoyne et al. 2018
AZA28	H	CH3	H	H	O							Kilkoyne et al. 2018
AZA29	H	H	CH3	H	H							Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H							Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H							Rehmann et al. 2008
AZA33	–	–	H	CH3	H							Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H							Kilkoyne et al. 2014
AZA35	–	CH3	H	CH3	H	CH3	CH3	830,5	362 262	A. spin/A. dexterop	phycotoxin	Kilkoyne et al. 2014, Rossi et al. 2017
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348 248	A. poporum	phycotoxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348 248	A. poporum	phycotoxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348 248	A. languida	phycotoxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348 248	A. languida	phycotoxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H	H	CH3	842,5	348 248	A. poporum	phycotoxin	Krock et al. 2014
AZA41	H	CH3	H	CH3	H							Krock et al. 2014
AZA42	OH	CH3	H	CH3	H							Krock et al. 2018
AZA43	–	H	H	CH3	H							Tillmann et al. 2017
AZA44	H	H	H	COOH	OH							Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH							Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH							Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH							Kilkoyne et al. 2015
AZA48	OH	H	H	H	H							Kilkoyne et al. 2018
AZA49	OH	CH3	H	H	H							Kilkoyne et al. 2018
AZA50	H	CH3	H	CH3	H							Tillmann et al. 2018
AZA51	OH	CH3	H	CH3	H							Tillmann et al. 2018
AZA52	nd	nd	nd	nd	nd							Tillmann et al. 2018
AZA53	nd	nd	nd	nd	nd							Tillmann et al. 2018
AZA54	nd	nd	nd	nd	nd							Rossi et al. 2017
AZA55	nd	nd	nd	nd	nd							Rossi et al. 2017
AZA56	nd	nd	nd	nd	nd							Rossi et al. 2017
AZA57	nd	nd	nd	nd	nd							Rossi et al. 2017
AZA58	nd	nd	nd	nd	nd	nd	nd	828,5	362 · 262	A. dexteroporum	Phycotoxin	Rossi et al. 2017
AZA59	OH	H	H	CH3	H	CH3	CH3	860,5	362 · 262	A. poporum	Phycotoxin	Kim et al. 2017
AZA60	H	H	H	OH	CH3	nd	nd	826,5	362 · 262	shellfish	metabolite	Kilkoyne et al. 2018
AZA61	H	CH3	H	OH	CH4	nd	nd	840,5	362 · 262	shellfish	metabolite	Kilkoyne et al. 2018
AZA62	nd	nd	nd	nd	nd	nd	nd	870,5	362 · 262	A. poporum	Phycotoxin	Krock et al. 2018

Currently
~70 published AZAs

Out of which
~34 AZAs are of
planktonic origin

At least additional
10 AZAs of bivalve
origin
(yet unpublished)

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Azaspiracids (AZA)

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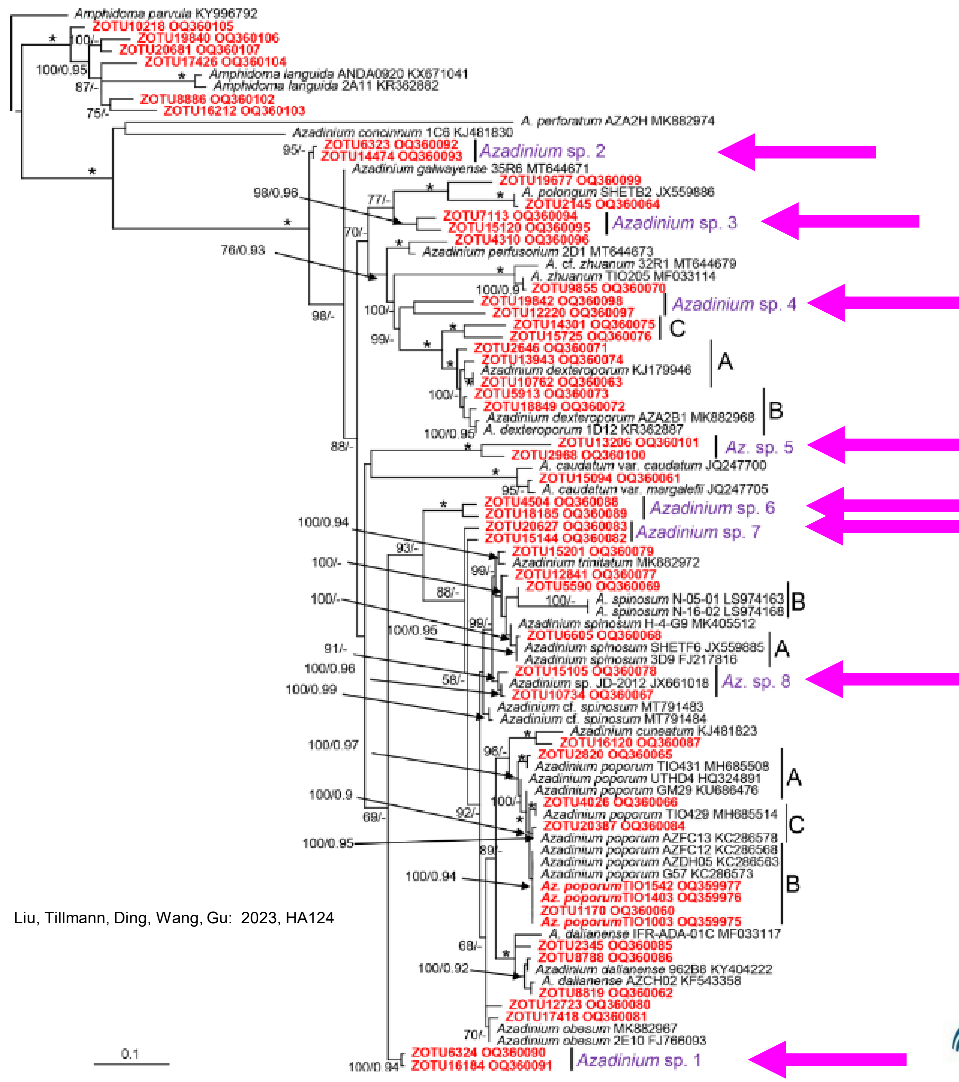
Yet unexplored diversity within Amphidomataceae

ITS based phylogenetic tree
of NGS field sample analyses
Chinese coastal waters



Liu, Tillmann, Ding, Wang, Gu: 2023, HA124

undescribe Azadinium taxa

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AZAs produced by algae[1]

AZA-1	AZA-41
AZA-2	AZA-42
epi-AZA-7	AZA-43
AZA-11	AZA-50
AZA-33	AZA-51
AZA-34	AZA-52
AZA-35	AZA-53
AZA-36	AZA-54
AZA-37	AZA-55
AZA-38	AZA-56
AZA-39	AZA-57
AZA-40	AZA-58
AZA-59	AZA-62

AZA shellfish metabolites of AZA-1 and -2

AZA-3	AZA-14	AZA-25	AZA-47
AZA-4	AZA-15	AZA-26	AZA-48
AZA-5+	AZA-16	AZA-27	AZA-49
AZA-6	AZA-17	AZA-28	AZA-60
AZA-7	AZA-18	AZA-29	AZA-61
AZA-8	AZA-19	AZA-30	
AZA-9	AZA-20	AZA-31	
AZA-10	AZA-21	AZA-32	
AZA-11*	AZA-22	AZA-44	
AZA-12	AZA-23	AZA-45	
AZA-13	AZA-24	AZA-46	

* Tillmann, U., et al. (2017) J Plankt Res 39(2), 350-367.
+ Montoya, N., pers. commun.

Krock, B., et al. (2019) Harmful Algae 82, 1-8.
[1] Additional planktonic AZA reported by Ozawa, M., et al. (2021) Toxicon 199: 145-155.

Two AZAs of phytoplankton origin result in 38 shellfish metabolites!

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Azaspiracids (AZA)

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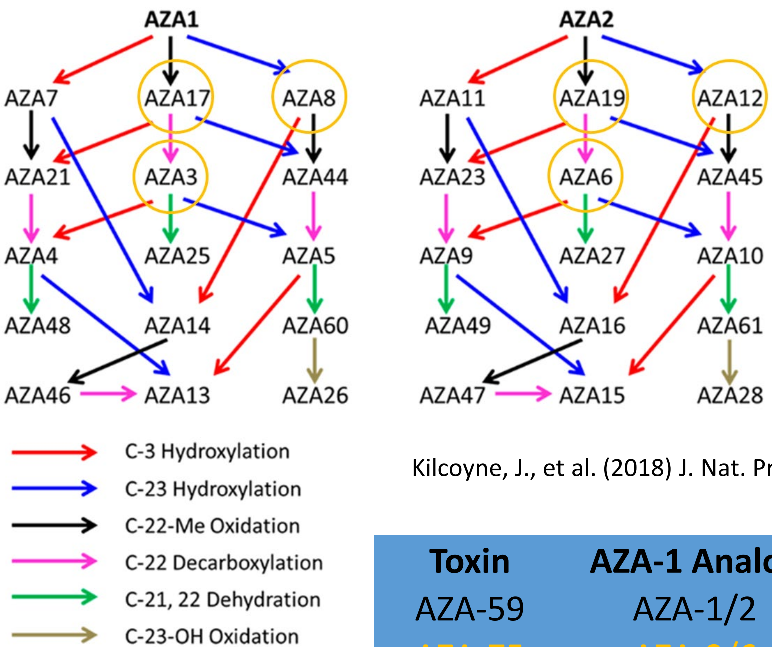
Azaspiracids – Metabolism in Bivalves



Kilcoyne, J., et al. (2018) J. Nat. Prod. 81(4), 885-893.

Toxin	AZA-1 Analog	m/z $[M+H]^+$	m/z $[M+H-H_2O]^+$	Retentiontime [min]
AZA-59	AZA-1/2	860	842	10.51
AZA-75	AZA-3/6	846	828	10.02
AZA-73	AZA-8/12	876	858	9.63
AZA-74	AZA-17/19	890	872	9.56
AZA-76	AZA-25/27	828	810	11.36
AZA-77	AZA-5/10	892	874	9.30
AZA-78	No analog	878	860	8.45

Krock, B., et al. in preparation

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Azaspiracids (AZA)

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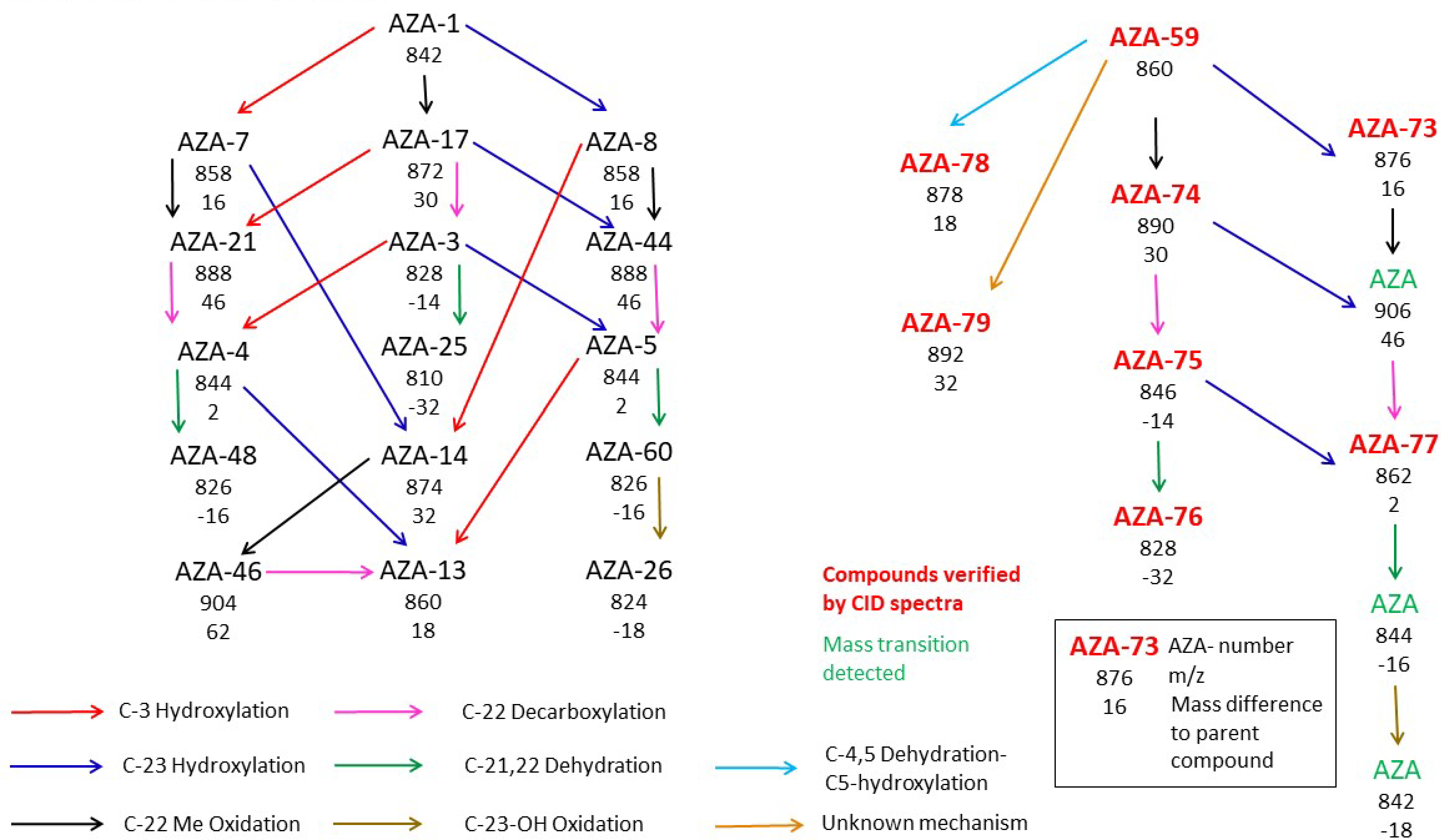
Azaspiracids – Metabolism in Bivalves

Kilcoyne, J., et al. (2018). J. Nat. Prod. 81(4): 885-893.



Azaspiracids (AZA)

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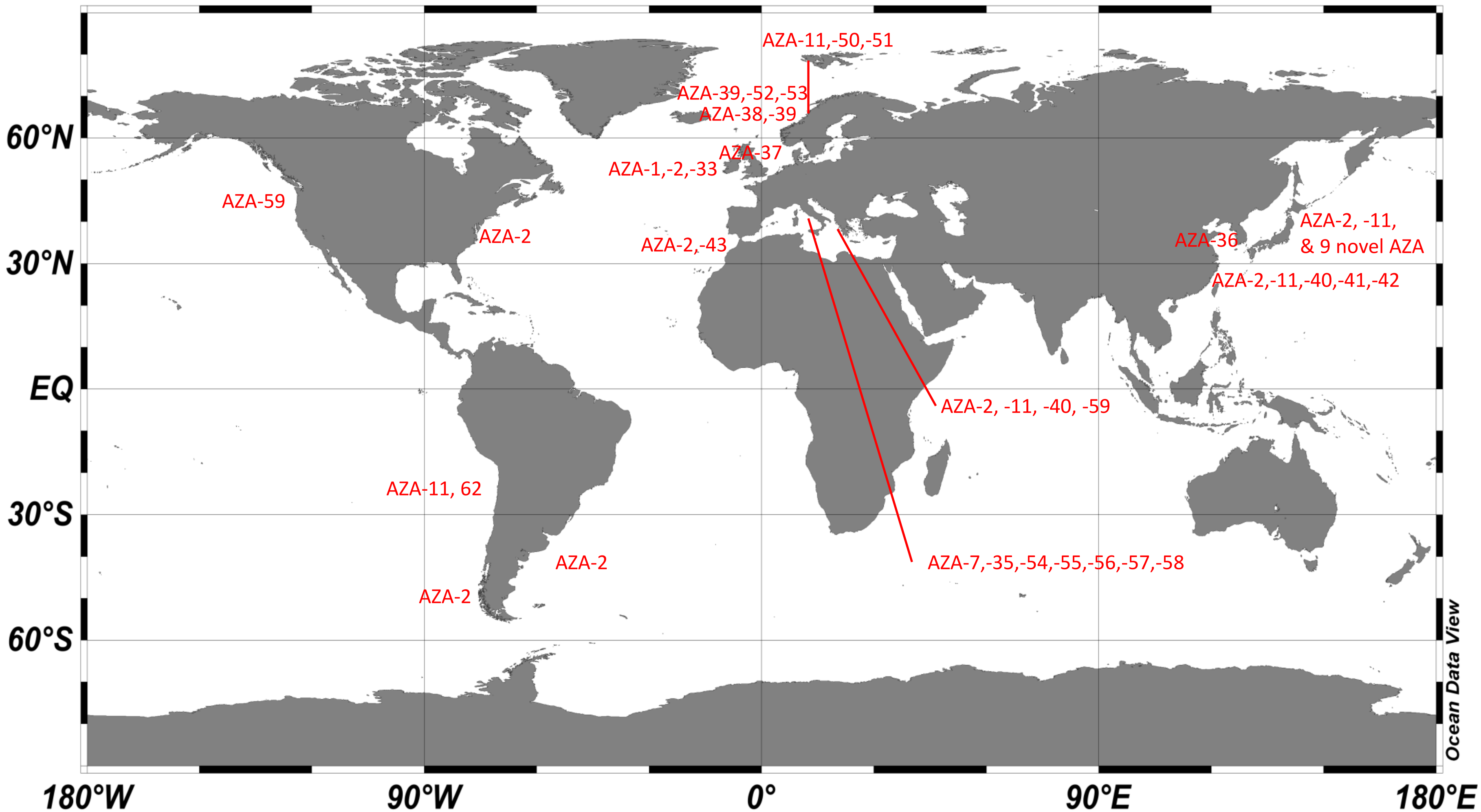
Azaspiracids – Geographic distribution



Ichthyotoxins

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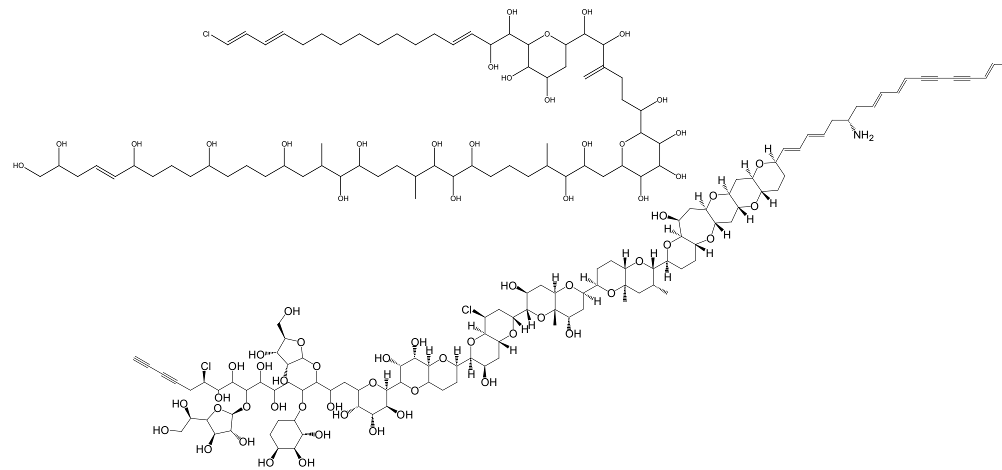
Variability

Amphidinols: 20+ known variants

Karlotoxins: 20+ known variants

Prymnesins: 100+ variants



Other ichthyotoxic species:

Alexandrium spp.
Chattonella spp.
Chrysocromulina spp.
Fibrocapsa japonica
Heterosigma akashiwo
Protoceratium reticulatum
Pseudochattonella cf. *verruculosa*

Ichthyotoxins:

Unknown !!

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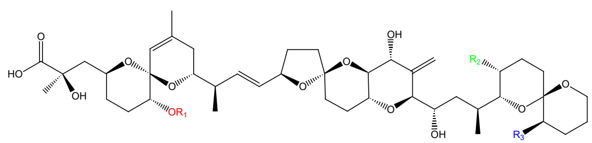
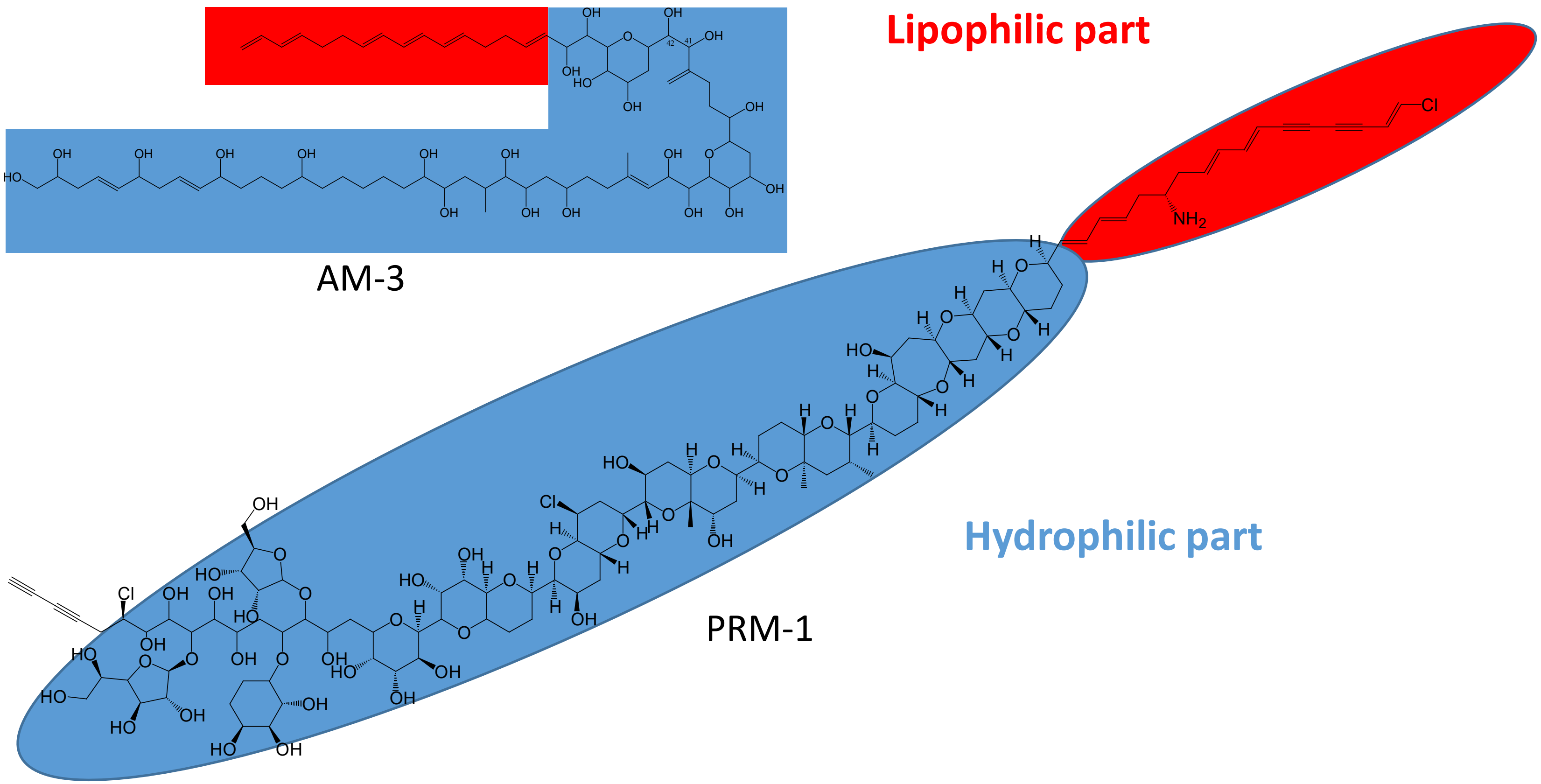
Ichthyotoxins

	Structure	Ecological function	Impact
Shellfish Poisoning Toxins	 √	?	Accumulation in the food chain, Intoxication of humans
Ichthyotoxins	?	Predation defense, Elimination of competitors √	Fish kills, Economic loss in aquacultures

Ichthyotoxins

Amphidinols – Ichthyotoxicity



Amphidinols (AM)

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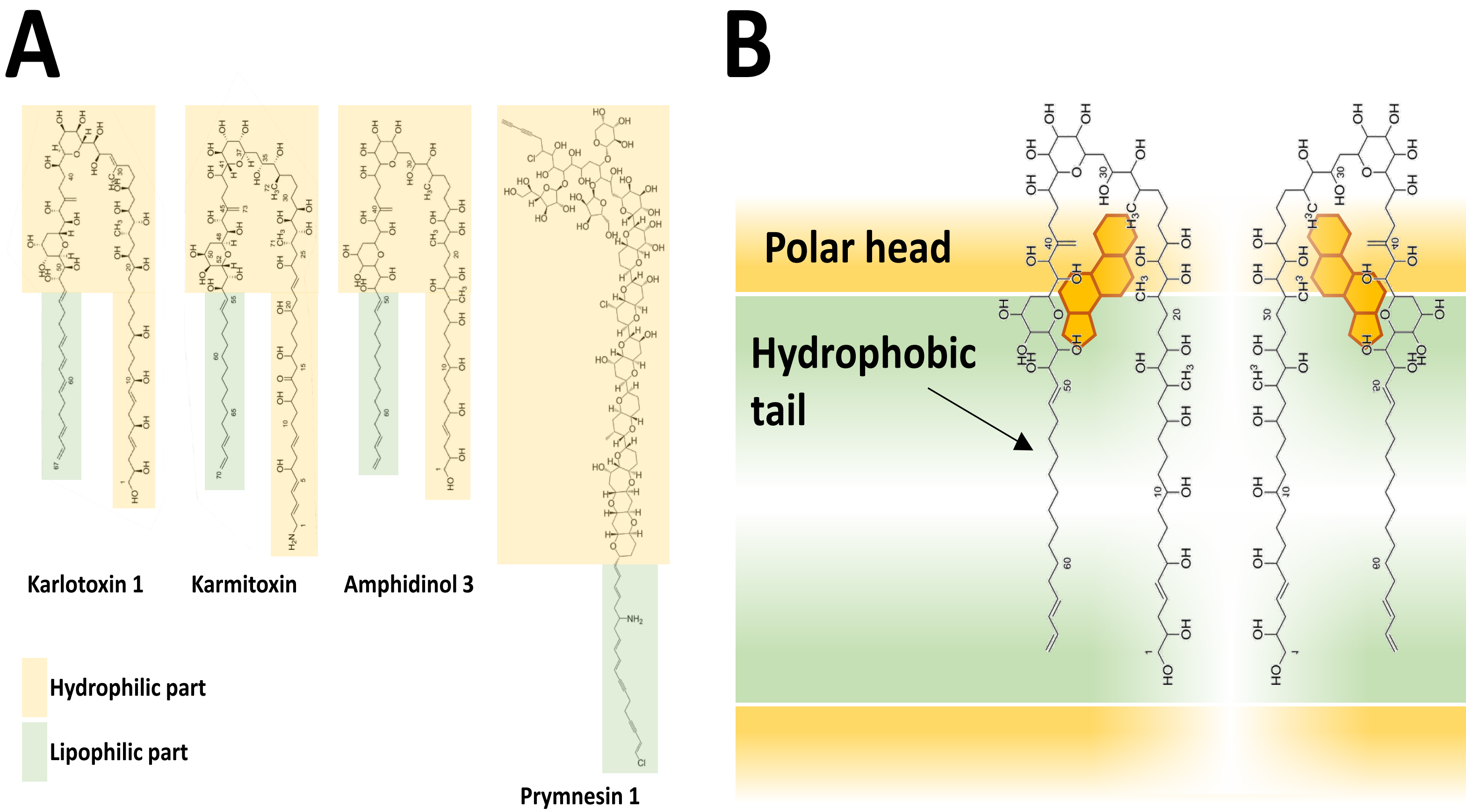
Mode of action of AM



Long, M., et al. (2021) Toxins 13(12): 905

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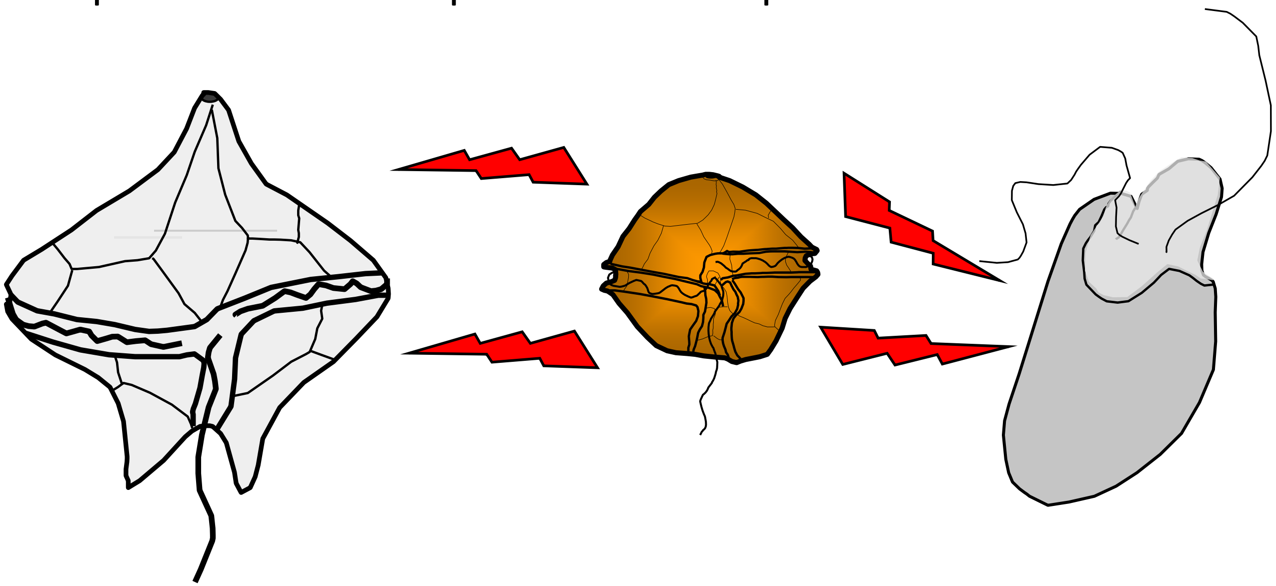
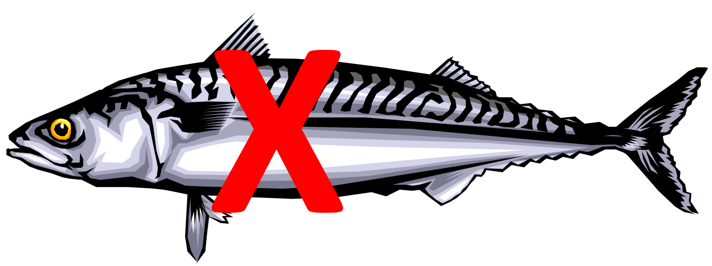
Ichthyotoxins

Current Hypothesis:

Lytic compounds of marine protists are weapons of chemical warfare among protists



Ichthyotoxicity is a collateral damage of protistan allelochemistry



Amphidinols (AM)

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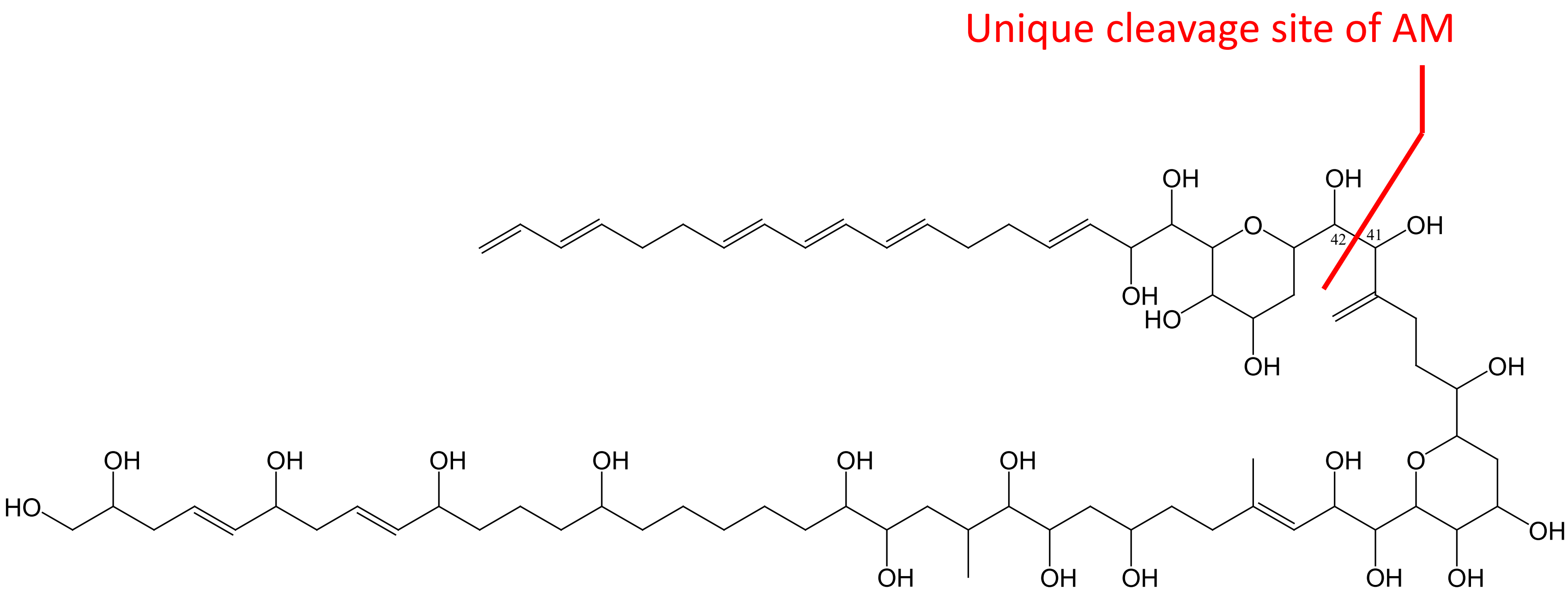
Structure of AM-3



$C_{70}H_{118}O_{23}$
Exact Mass: 1326,8064
C, 63,33; H, 8,96; O, 27,72

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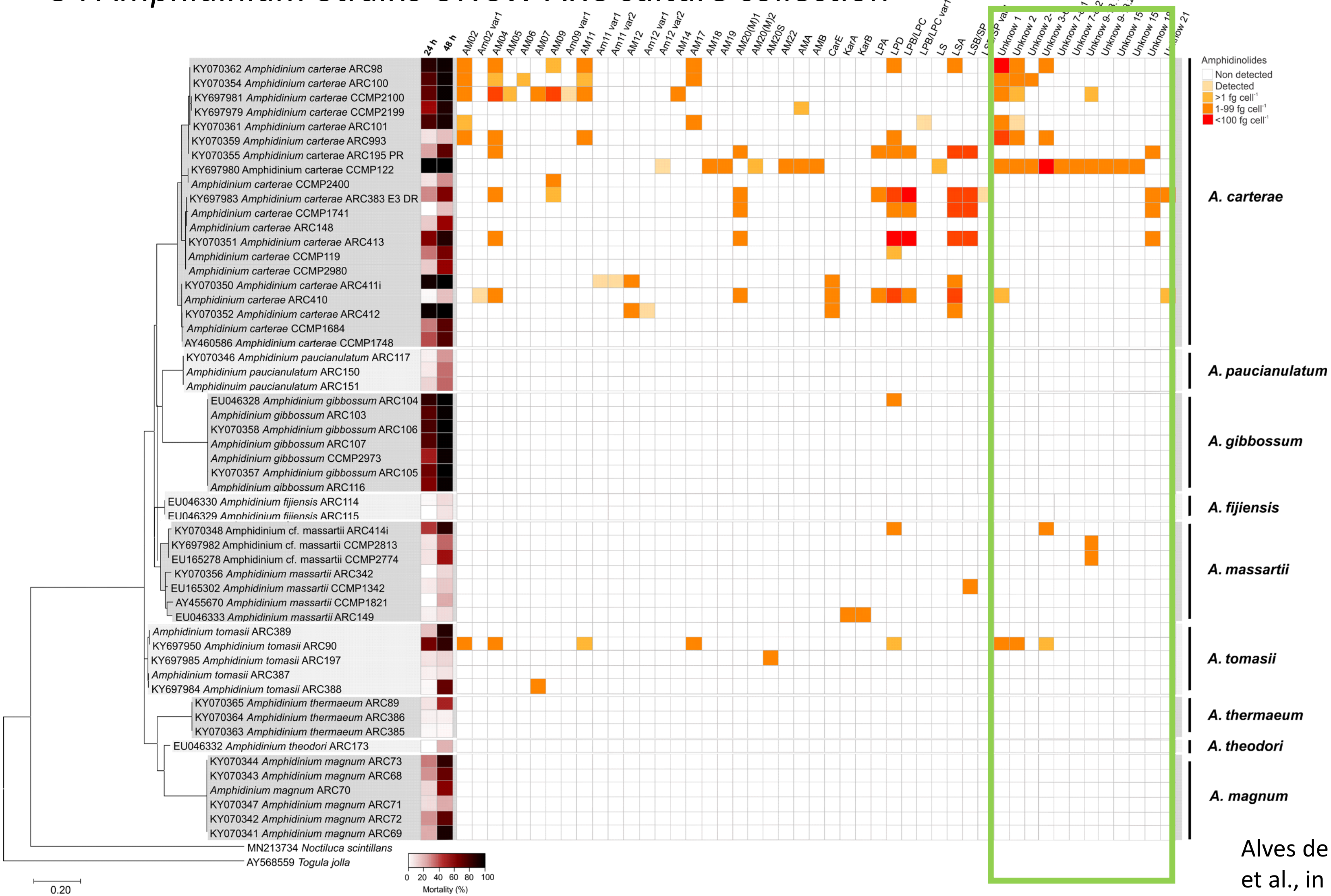
Amphidinols (AM)

54 *Amphidinium* Strains UNCW ARC culture collection



Alves de Souza, C., et al., in prep.

Take Home Messages

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1) Phycotoxin variability of known toxin classes is high and yet not fully explored

2) Toxins from planktonic origin are modified by vector organisms and futher augment chemical variabilty of toxins

3) Phycotoxin profiles can be geographically very variable

4) Ichthyotoxins are rarely characterized, but pose an increasing threat due to increasing aquacultural activities

5) Regulated toxins do not reflect levels of actual toxin content

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Any
Questions?
Thanks for
Your Attention!

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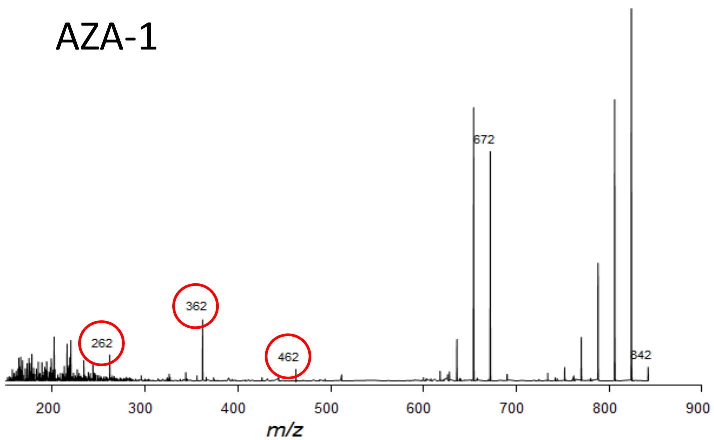
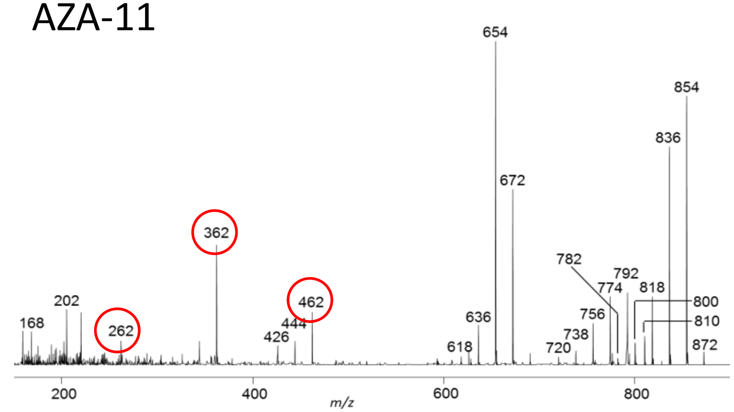
Product Ion Spectra of Azaspiracids

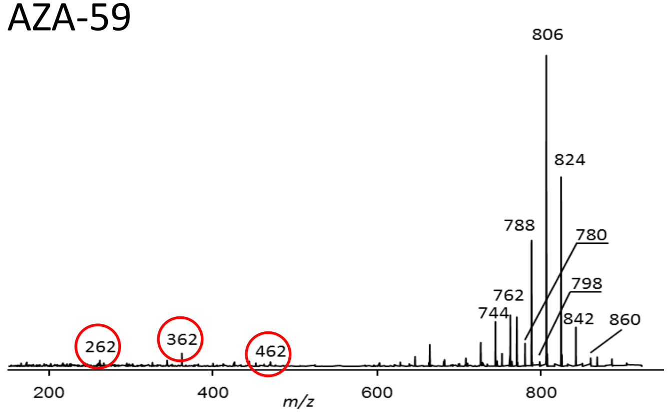
AZA-1



AZA-11



AZA-59



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