

Examples of Structural Variability of Phycotoxins and

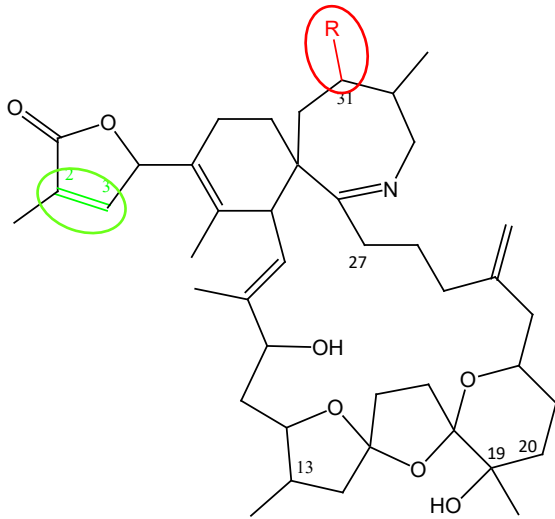
Implications for Monitoring and Regulation

Outline

1. Cyclo imines
2. Goniodomins
3. Karlotoxins
4. DSP
5. AZP
6. Discrete vs. integrated monitoring

1. Cyclo imines

Spirolides – Chemical Structures



Spirolide A: **R = H**, $\Delta^{2,3}$

B: **R = H**

C: **R = Me**, $\Delta^{2,3}$

D: **R = Me**

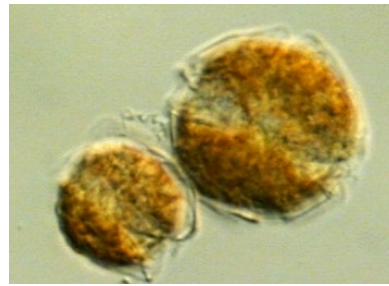
27-hydroxy-13-desmethyl-SPX C

27-hydroxy-13,19-didesmethyl-SPX C

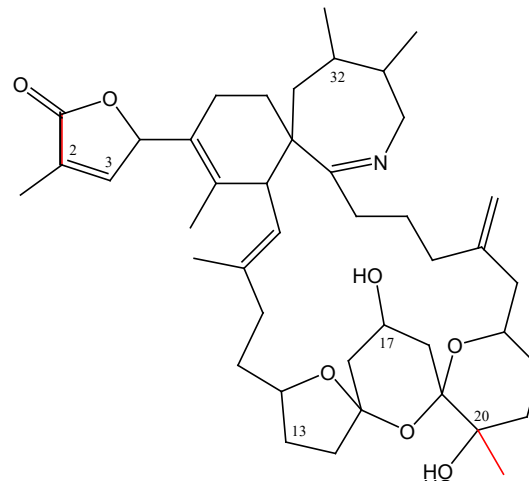
27-oxo-13,19-didesmethyl-SPX C

20-hydroxy-13,19-didesmethyl-SPX C

20-hydroxy-13,19-didesmethyl-SPX D

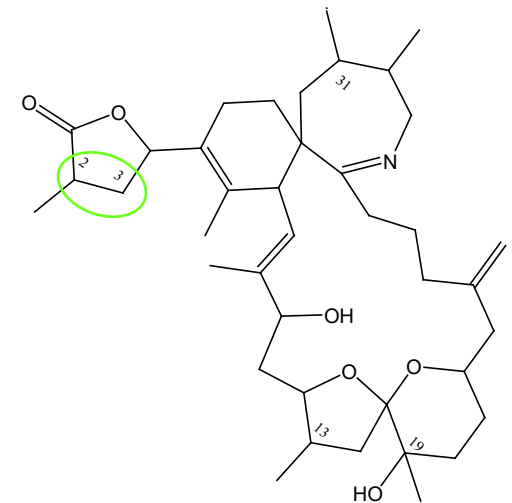


Alexandrium ostenfeldii



SPX G

20-methyl-SPX G

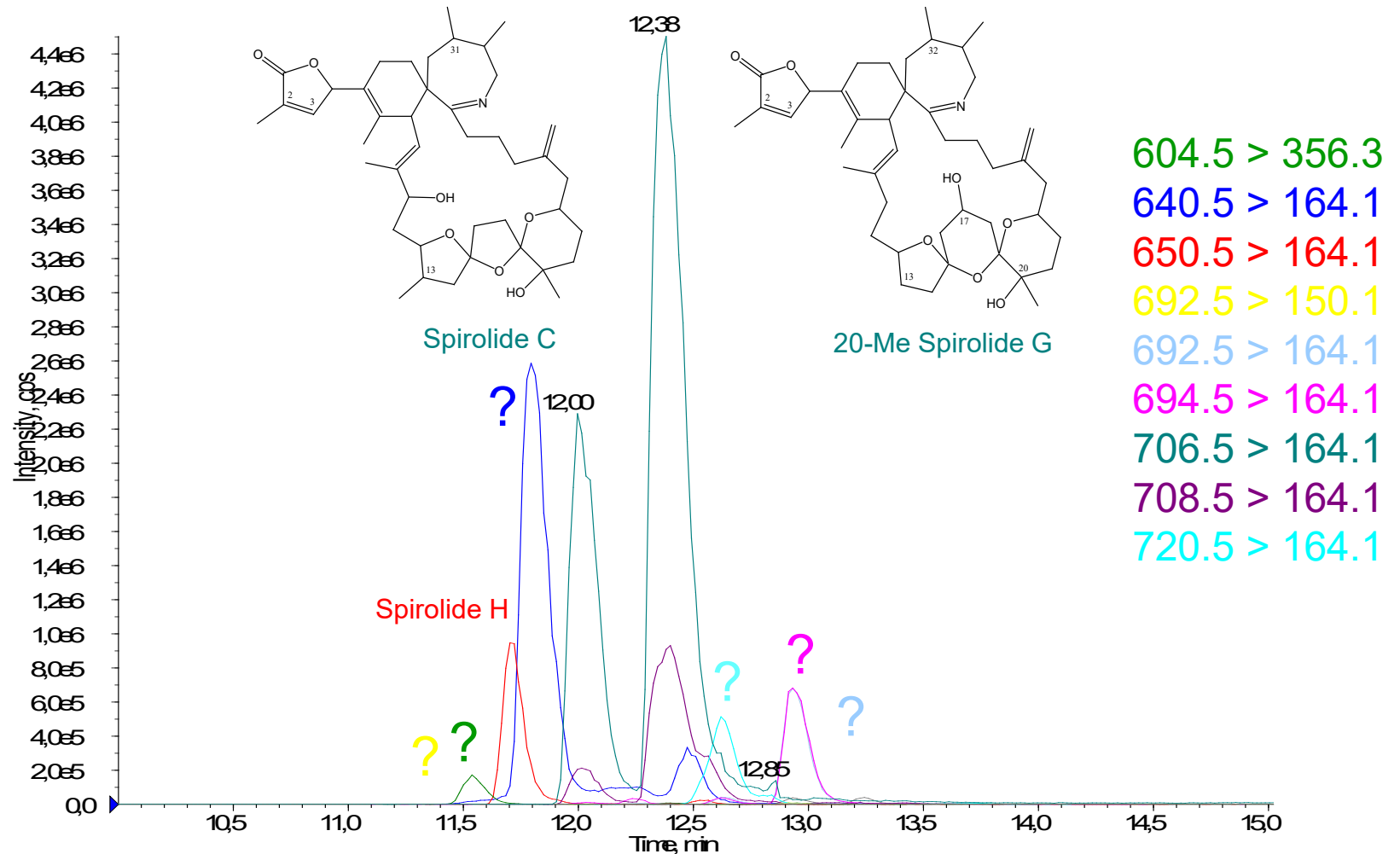


SPX H

SPX I

1. Cyclo imines

Alexandrium ostenfeldii (AOSH2, Nova Scotia, Canada)



1. Cyclo imines

Alexandrium ostenfeldii (Disko Island, Greenland)

	Stat	SPX-1	C	20-meG	H	Cp 1	Cp 2	Cp 3	Cp 4	Cp 5	Cp 6	Cp 7	Cp 8
P1 D5	506	-	77.1	16.8	-	-	-	-	-	-	3.4	2.7	-
P1 H10	516	0.7	-	84.3	-	0.1	27.5	0.3	-	0.8	-	-	13.7
P2 E3	516	31.2	-	-	41.3	-	-	-	-	-	-	-	-
P2 E4	516	19.2	-	-	-	1.2	-	-	-	70.1	-	-	9.4
P2 F2	516	5.1	63.6	-	6.7	0.1	4.1	-	-	18.6	-	3.8	-
P2 F3	516	-	82.9	17.1	-	-	-	-	-	-	-	-	-
P2 F4	516	2.7	57.3	39.7	-	-	-	-	-	-	0.1	-	-
P2 F7	516	1.4	31.1	-	25.1	1.2	10.0	-	-	29.0	-	-	2.3
P2 G2	516	0.2	40.2	18.4	7.1	-	11.1	-	-	11.6	-	-	11.3
P2 G9	516	-	100.0	-	-	-	-	-	-	-	-	-	-
P2 H4	516	-	95.4	4.6	-	-	-	-	-	-	-	-	-
P2 H8	516	2.4	-	89.0	-	-	-	-	-	0.3	-	-	8.2
P3 F1	516	0.2	-	81.7	-	-	0.1	0.2	-	1.0	-	-	16.9
P4 C6	516	-	50.3	49.7	-	-	-	-	-	-	-	-	-
P4 E3	516	1.2	96.3	2.2	-	-	-	8.9	-	-	-	-	-
P4 D8	516	-	31.4	-	14.1	-	-	9.0	36.1	9.4	-	-	-
P4 F10	516	-	68.6	20.0	0.2	-	-	-	-	-	-	-	11.3
P4 G2	516	-	-	100.0	-	-	-	-	-	-	-	-	-
P3 A12	516	-	52.2	-	47.8	-	-	-	-	-	-	-	-
P2 H2	516	-	-	92.5	-	-	-	0.9	-	-	-	-	6.6
P2 G3	516	18.1	1.3	0.1	-	-	-	-	-	72.7	-	-	7.8
P3 E4	516	0.2	99.6	-	-	-	-	0.2	-	-	-	-	-
P4 F4	516	-	77.4	19.6	0.3	-	-	0.4	-	2.1	-	-	-
P1 F5	524	0.1	79.5	19.8	-	-	-	0.2	-	-	0.1	0.2	-
P1 F7	524	0.1	78.3	20.9	-	-	0.1	0.4	-	-	0.1	-	-
P1 F8	524	-	92.1	6.6	-	-	0.1	0.4	-	-	0.2	0.5	-
P1 F9	524	0.2	64.7	33.2	-	-	0.1	1.4	-	-	0.3	-	-
P1 F10	524	0.1	68.4	30.5	-	-	0.1	0.9	-	-	0.1	-	-
P1 F11	524	-	87.3	6.4	-	-	-	-	-	-	-	5.4	-
P1 G3	524	0.6	92.3	6.6	-	-	-	0.6	-	-	-	-	-
P1 G5	524	0.7	76.1	21.7	0.4	-	-	0.4	-	-	0.3	0.4	-
P1 G11	524	0.2	84.2	14.2	0.5	-	-	0.3	-	-	0.3	0.3	-
P1 G8	524	-	88.6	6.5	-	-	-	-	-	-	-	4.9	-
P1 F6	524	0.1	85.1	13.8	-	-	0.1	0.1	-	-	0.1	0.3	-
P1 F4	524	0.1	77.2	22.3	-	-	-	-	-	-	0.1	0.2	-
P1 G6	524	-	70.5	28.0	0.3	-	-	0.6	-	-	0.2	0.3	-

high toxin
variability
within
populations

Tillmann, U., et al. (2014)
Harmful Algae 39, 259-270.

1. Cyclo imines

Cyclic imines – Chemical Variability

Known Variants

Mass transition	toxin
508>490	GYM A
510>492	16-desme GYM D
522>504	12-me GYM A
524>506	GYM B/C/D
526>508	GYM E
650>164	H
652>164	I
678>164	13,19-didesme C
692>164	13-desme C, G, undescribed
692>178	27-oxo-13,19-didesme C
692>150	A, undescribed
694>164	13-desme D, 20-hydroxy.-13,19-didesme C, pinnatoxin G
694>180	27-hydroxy-13,19-didesme C
694>150	B
696>164	20-hydroxy-13,19-didesme D
706>164	C, 20-me G
706>164	27-hydroxy-13-desme C
708>164	D
766>164	pinnatoxin F
784>164	pinnatoxin E

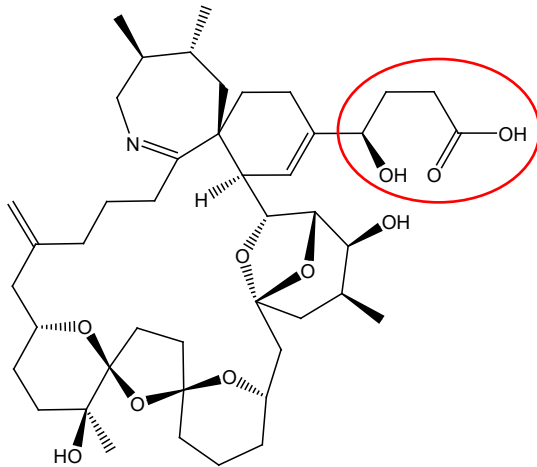
Undescribed Variants

Mass transition	spirolide
640>164	undescribed
644>164	undescribed
658>164	undescribed
658>150	undescribed
674>164	undescribed
678>150	undescribed
692>150	A, undescribed
696>164	undescribed
698>164	undescribed
710>164	undescribed
710>150	undescribed
720>164	undescribed
722>164	undescribed
722>180	undescribed

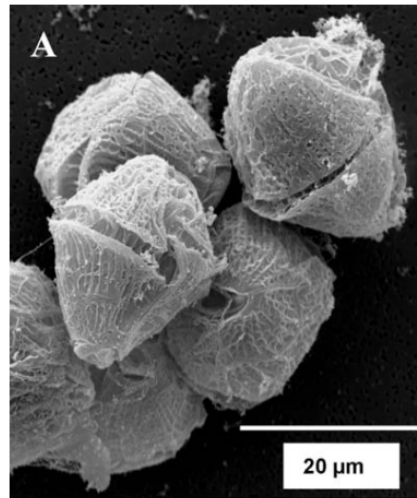
Tillmann, U., et al. (2014) Harmful Algae 39, 259-270
Zurhelle, C., et al. (2018) Marine Drugs 16(11).

1. Cyclo imines

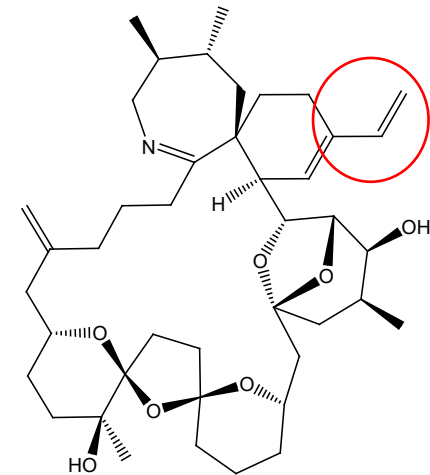
Pinnatoxins – structures



Pinnatoxin E

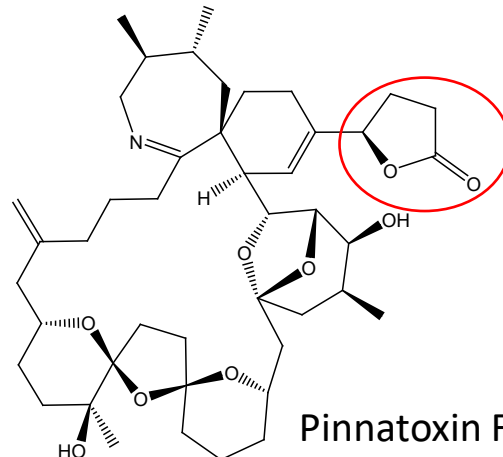


Rhodes, L., et al., 2010, Harmful Algae, 9, 384-389.



Pinnatoxin G

*Vulcanodinium
rugosum*

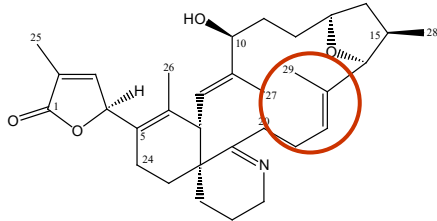


Pinnatoxin F

Rhodes, L., et al. (2011) N.Z. J. Mar. Freshwater Res. 45(4): 703-709.

1. Cyclo imines

Gymnodimines - Structures

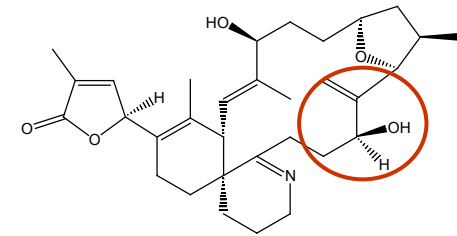


Gymnodimine A

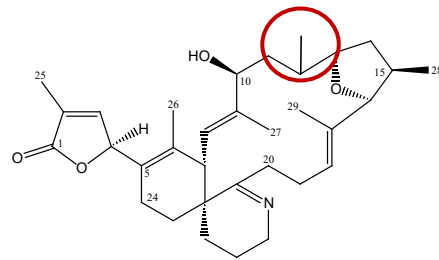


*Karenia
selliformis*

www.inaturalist.org/taxa/470162-Karenia-selliformis



Gymnodimine B/C

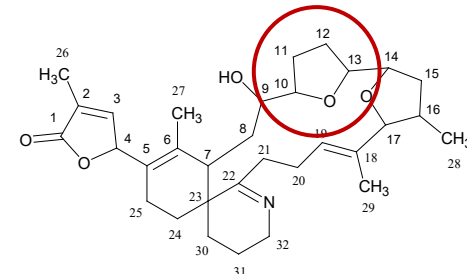


12-methyl-Gymnodimine A

Van Wagoner, R.M., et al. (2011)
Tetrahed. Lett. 52(33), 4243-4246.

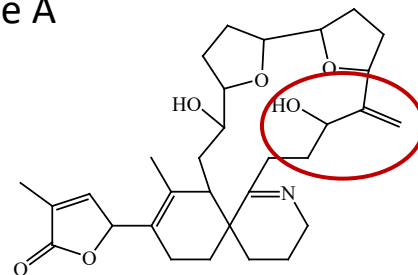


Alexandrium ostenfeldii



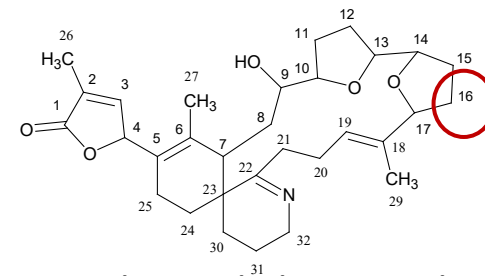
Gymnodimine D

Harju, K., et al. (2016) Toxicon 112, 68-76.



Gymnodimine E

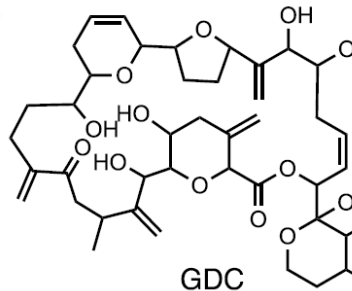
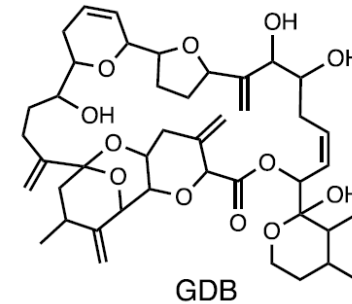
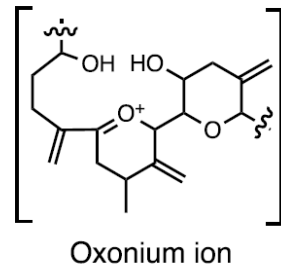
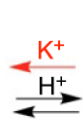
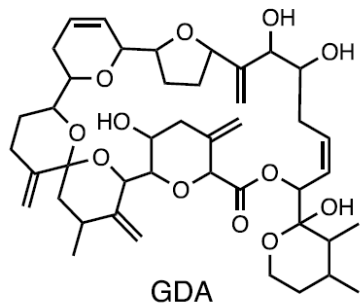
Zurhelle, C., et al. (2018) Marine Drugs 16(11).



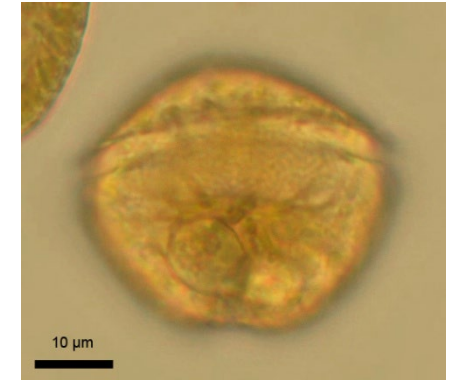
16-desmethyl-Gymnodimine D

2. Goniodomins

Goniodomins – Structures



Harris, C.M., et al. (2021). *J. Nat. Prod.* 84(9): 2554-2567.



*Alexandrium
pseudogonyaulax*

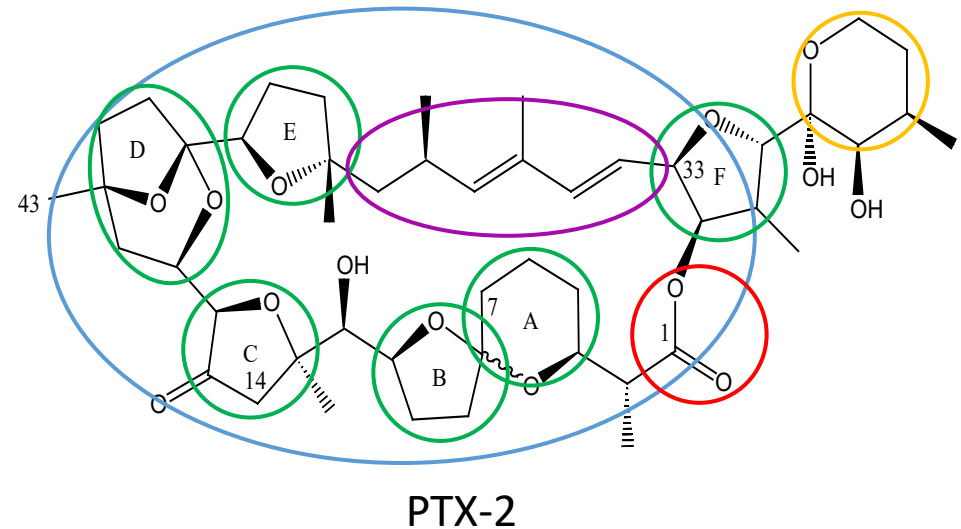
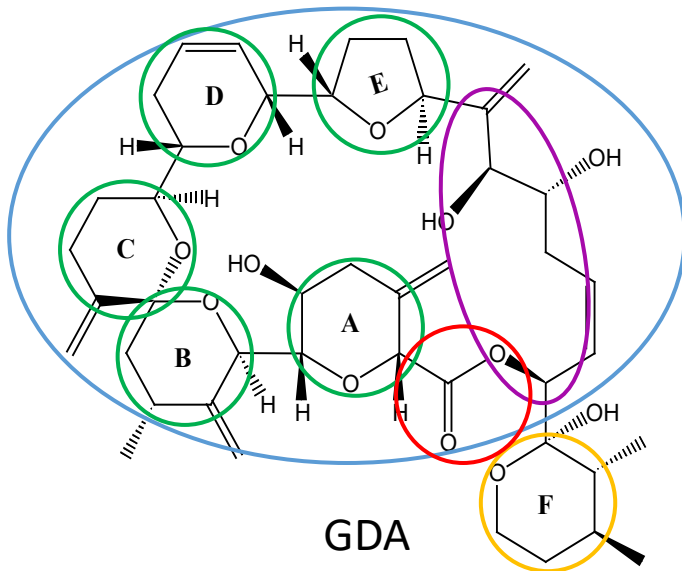
Producing species:

*Alexandrium
monilatum
pseudogonyaulax
hiranoi
taylorii*

Tillmann, U., et al. (2020). *Toxins* 12(9): 564.

2. Goniodomins

Goniodomins – Structural characteristics



Macrocyclic, exocyclic ether ring, ether rings, 6-membered carbon chain, lactone function

Toxicity: unknown

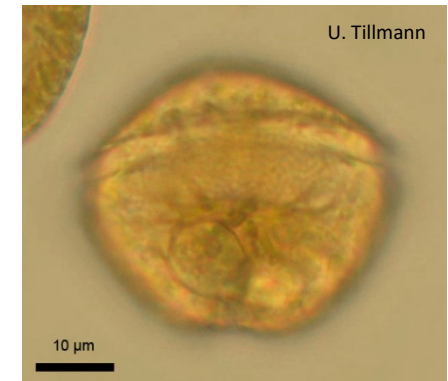
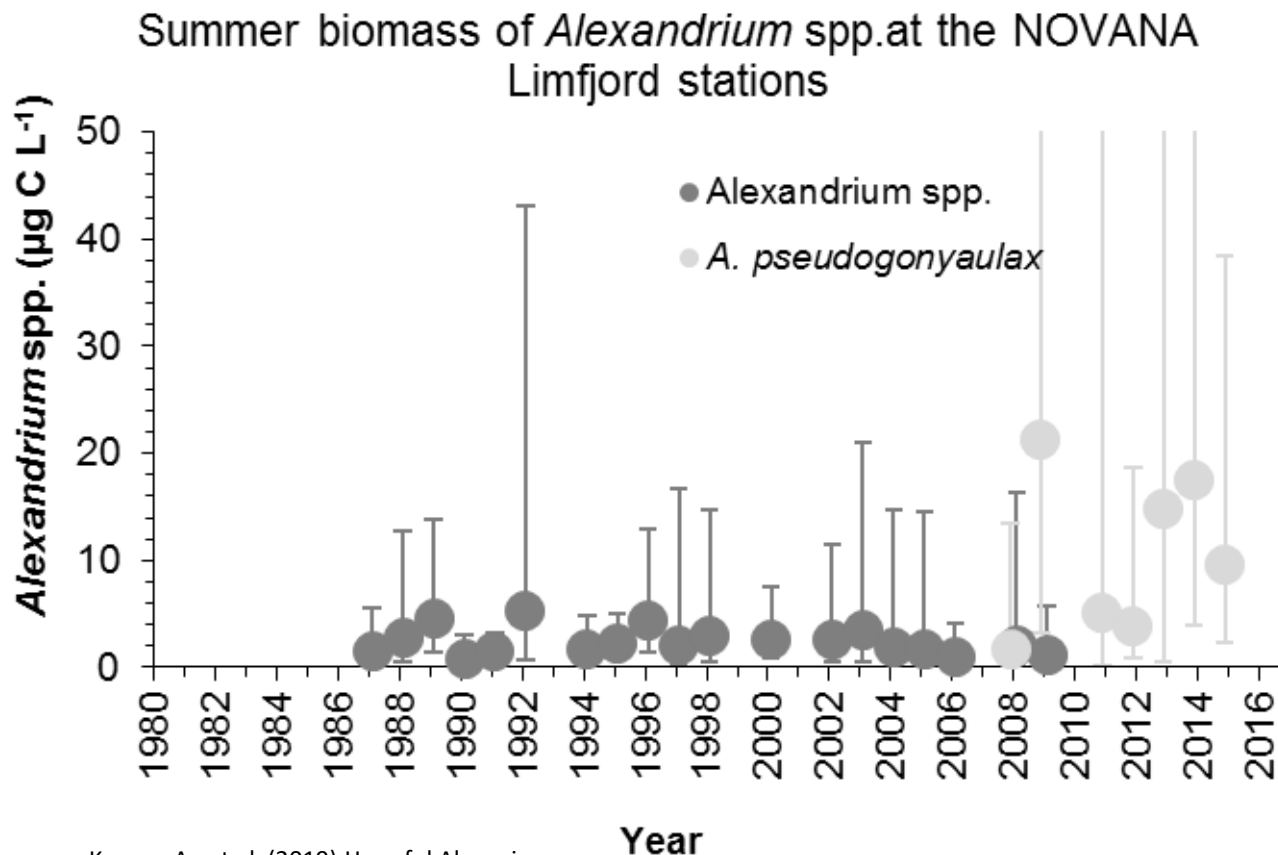
hepatotoxic

Due to the structural similarity of GDA and pectenotoxins (PTX), related toxicological effects can be assumed.

2. Goniodomins

Goniodomins – Community change

Community shift from an *Alexandrium catenella/ostenfeldii* to *A. pseudogonyaulax*

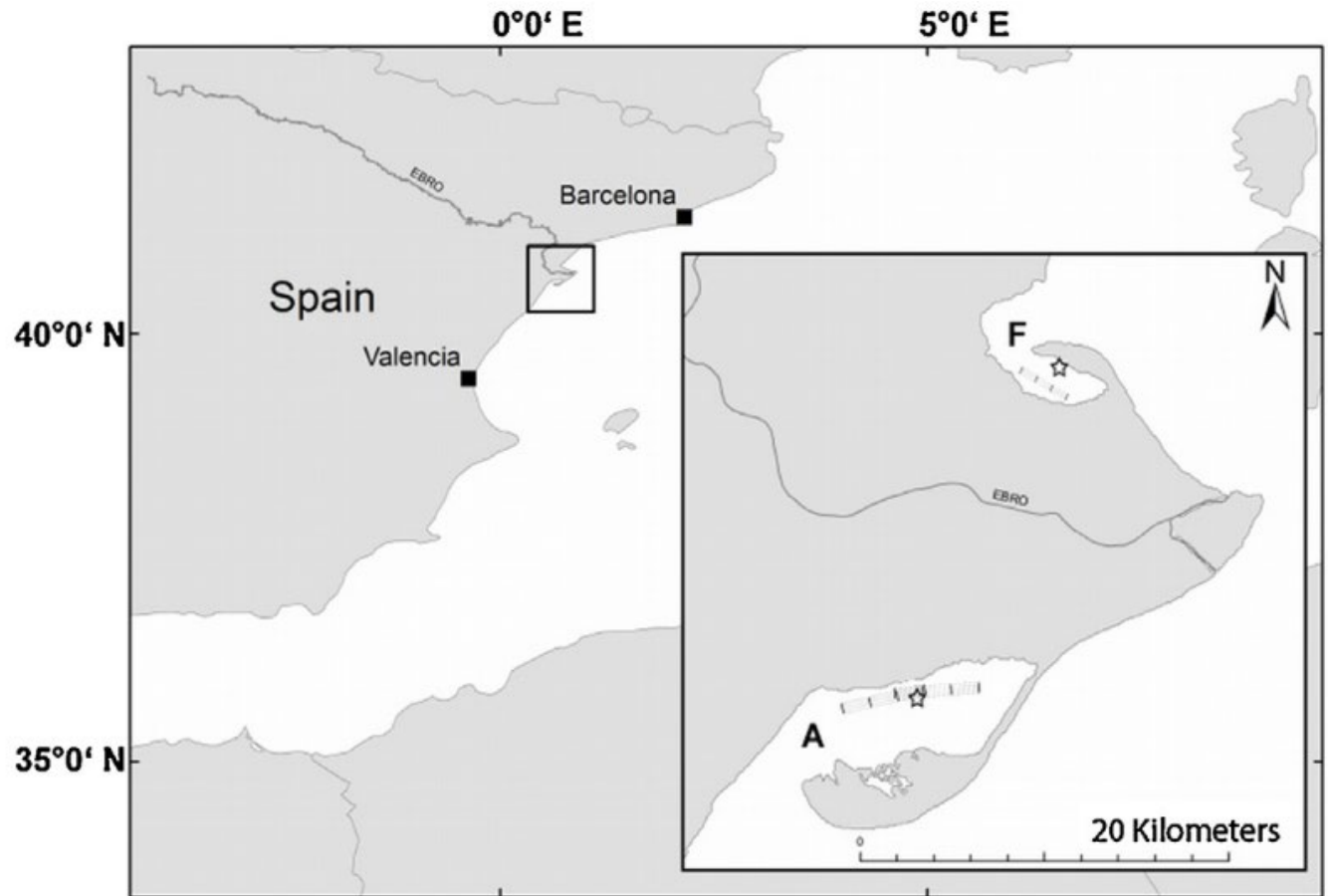


Alexandrium pseudogonyaulax

Producer of goniodomins

Kremp, A., et al. (2019) Harmful Algae, in press

3. Karlotoxins



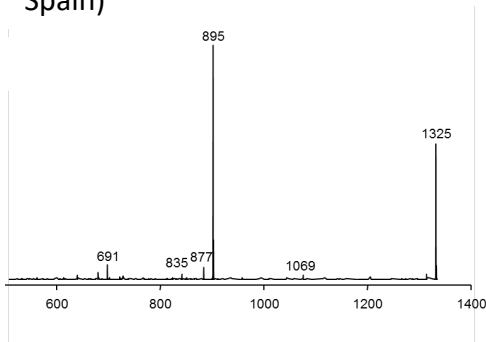
F = Fangar Bay

A = Alfacs Bay

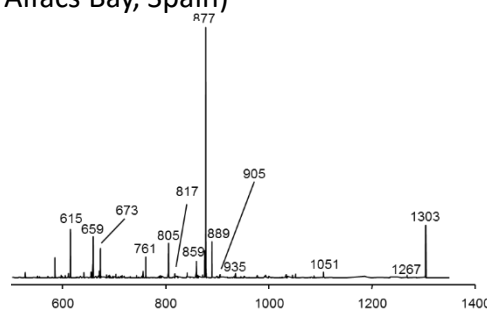
Busch, J.A., et al. (2016) Harmful Algae 55, 191-201.

3. Karlotoxins

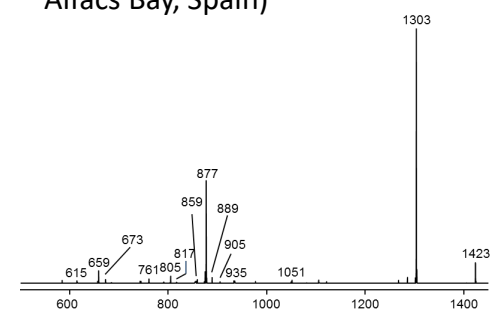
KmTx-5 (Strain E11, Fangar Bay, Spain)



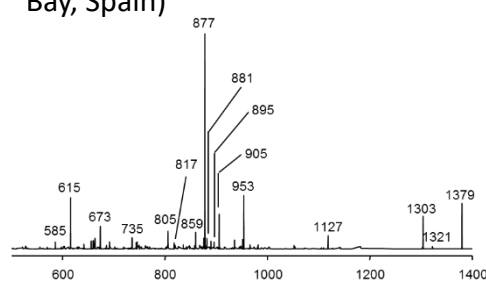
KmTx-10 (Strain K10, Alfacs Bay, Spain)



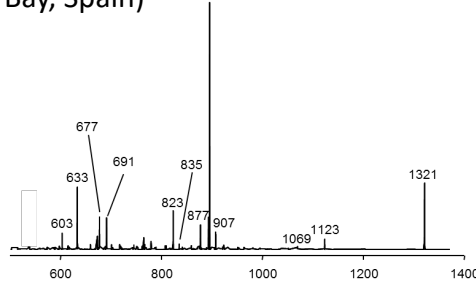
sulfo-KmTx-10 (Strain K10, Alfacs Bay, Spain)



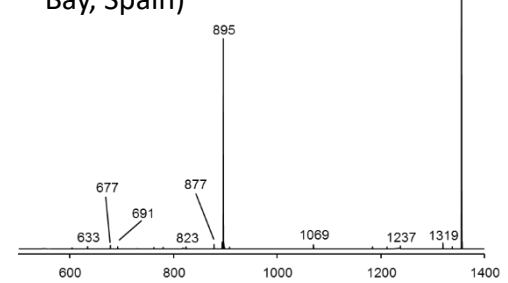
KmTx-11 (Strain K10, Alfacs Bay, Spain)



KmTx-12 (Strain K10, Alfacs Bay, Spain)



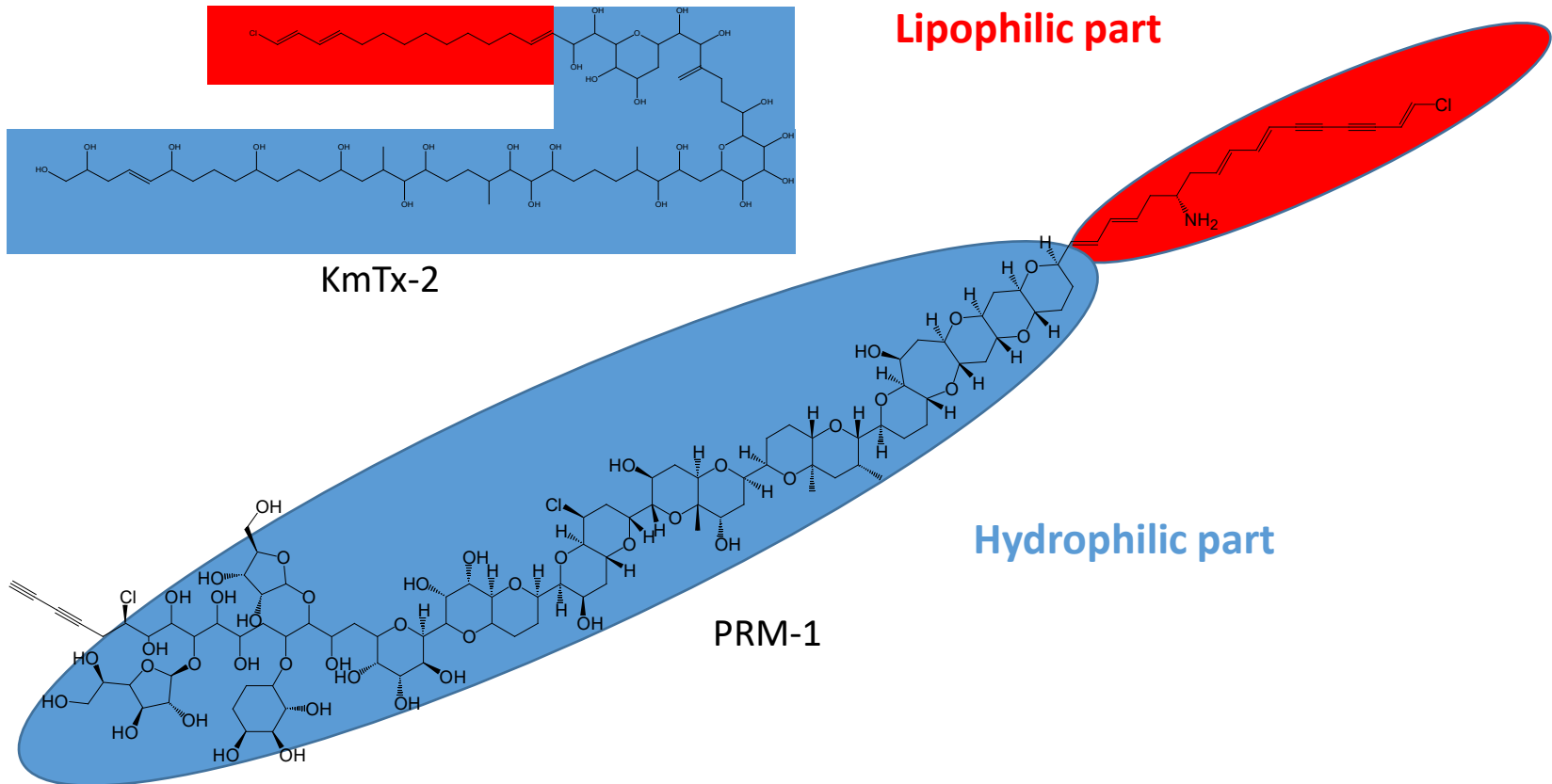
KmTx-13 (Strain K10, Alfacs Bay, Spain)



Krock, B., et al. (2017) Marine Drugs 15(12), 1-20.

3. Karlotoxins

Karlotoxins – Ichthyotoxicity



3. Karlotoxins

Ichthyotoxins – 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 c.f. *veruculosa*

**Ichthyotoxins:
Unknown !!**

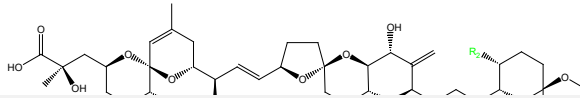
3. Karlotoxins

Ichthyotoxins – Variability

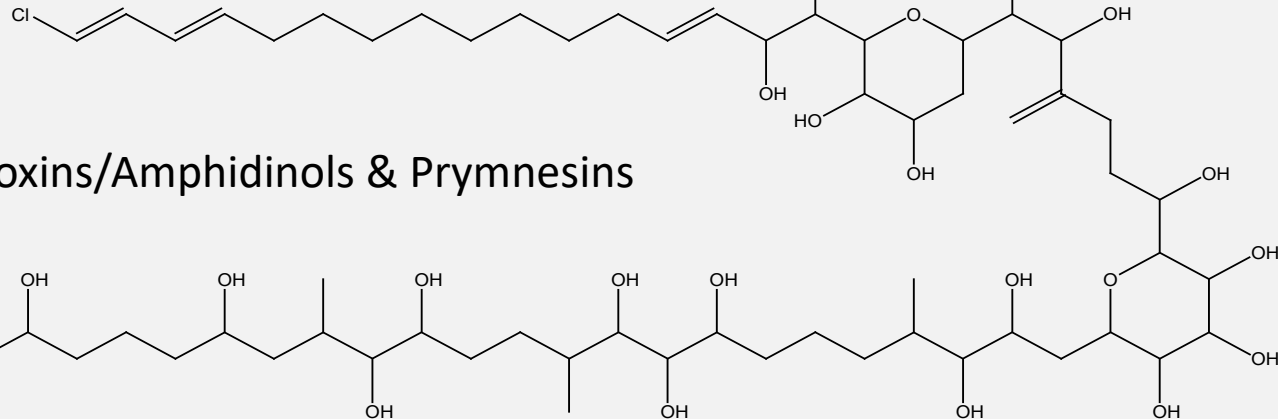
Structure

Ecological function

Impact



Accumulation in

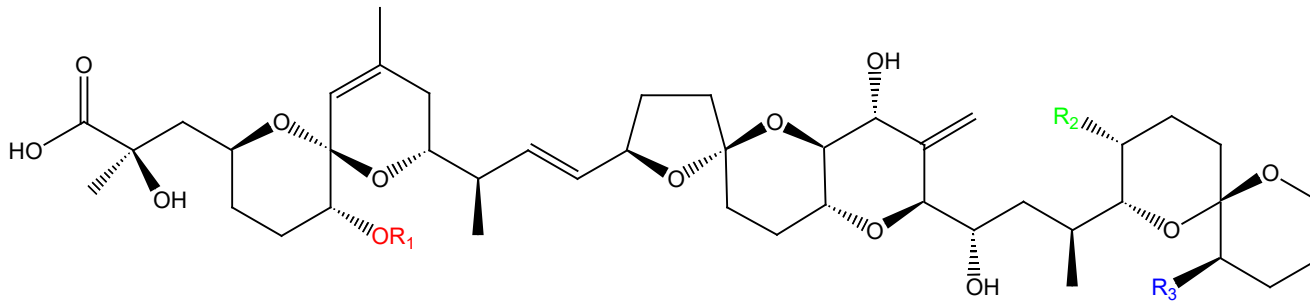


Karlotoxins/Amphidinols & Prymnesins

aquacultures



4. DSP Toxins

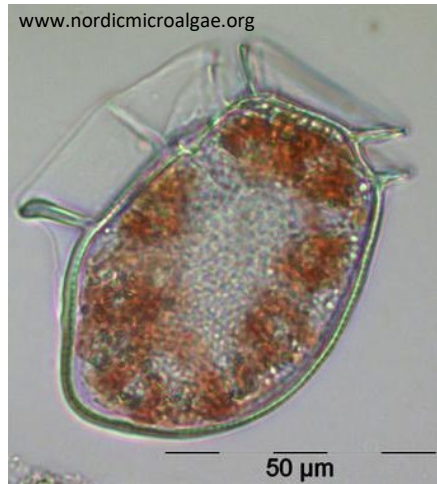


	R1	R2	R3
OA	H	CH ₃	H
DTX1	H	CH ₃	CH ₃
DTX2	H	H	CH ₃
DTX3	CH ₃ CO	CH ₃	CH ₃
Acyl-OA	CH ₃ CO	CH ₃	H

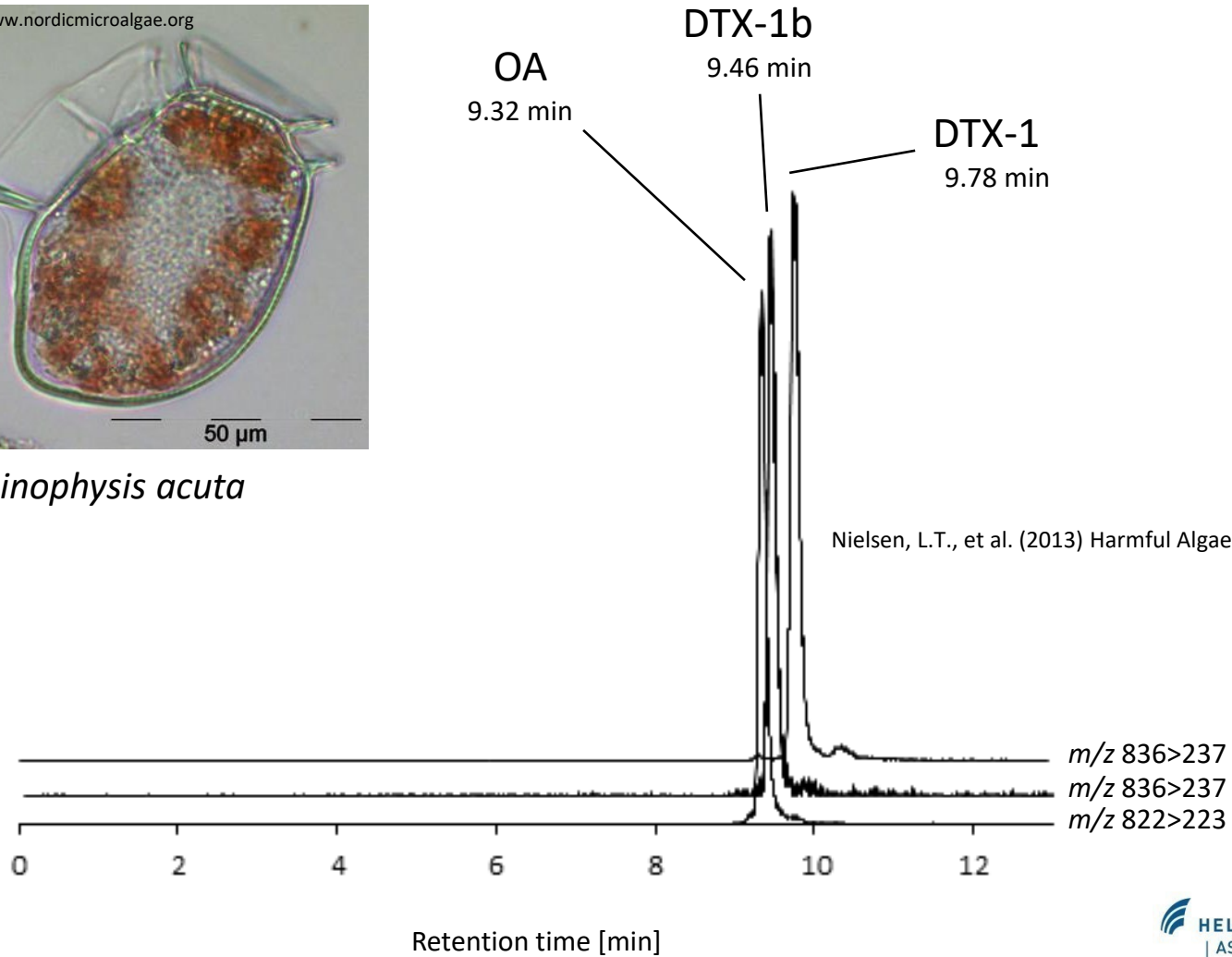
Dinophysis spp.
Prorocentrum spp.

OA: Okadaic acid (first isolated from the sponge *Halichondria okadai*)

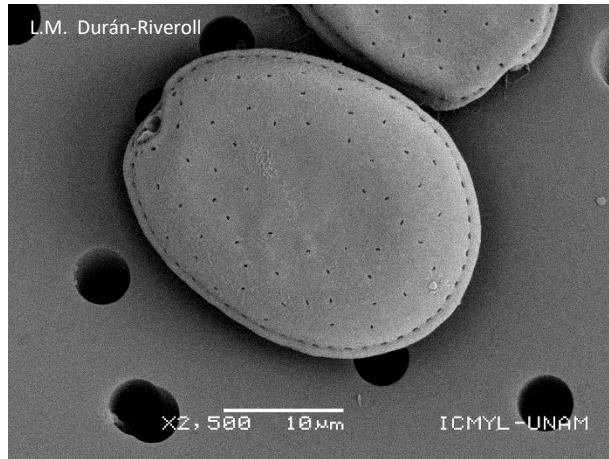
4. DSP Toxins



Dinophysis acuta

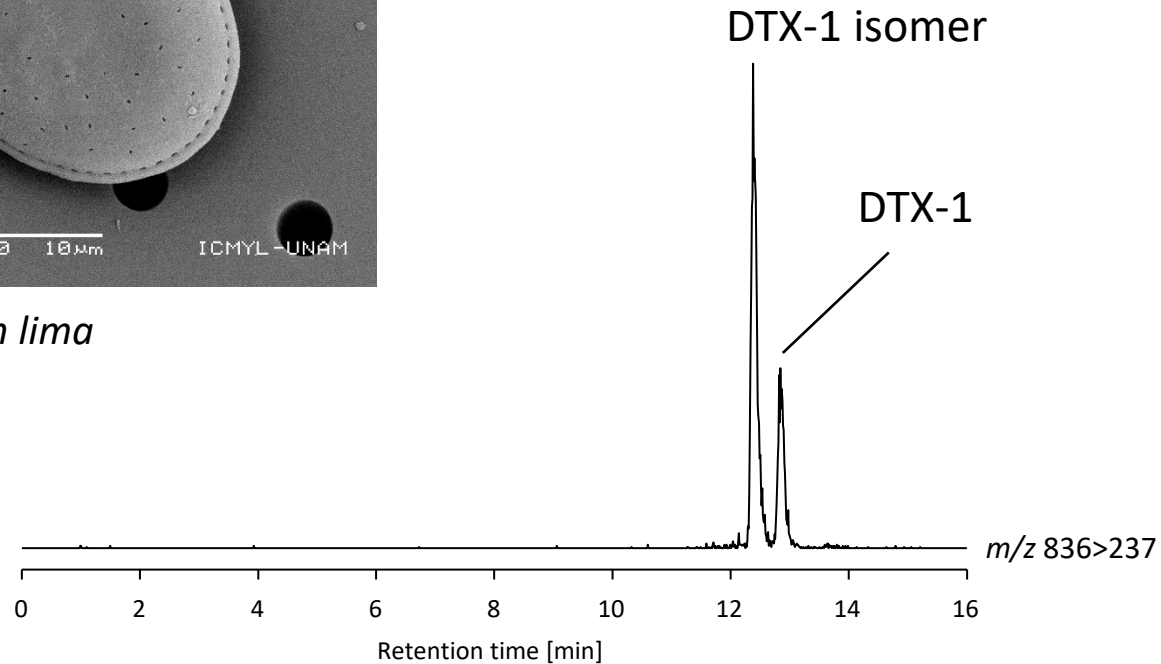


4. DSP Toxins



Prorocentrum lima

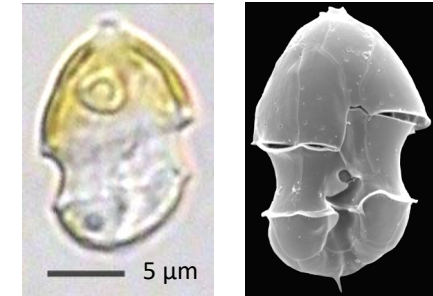
Prorocentrum lima, Mexico



Tarazona-Janampa U.I. et al., (2020). *Frontiers in Marine Science* 7(569).

5. AZP Toxins

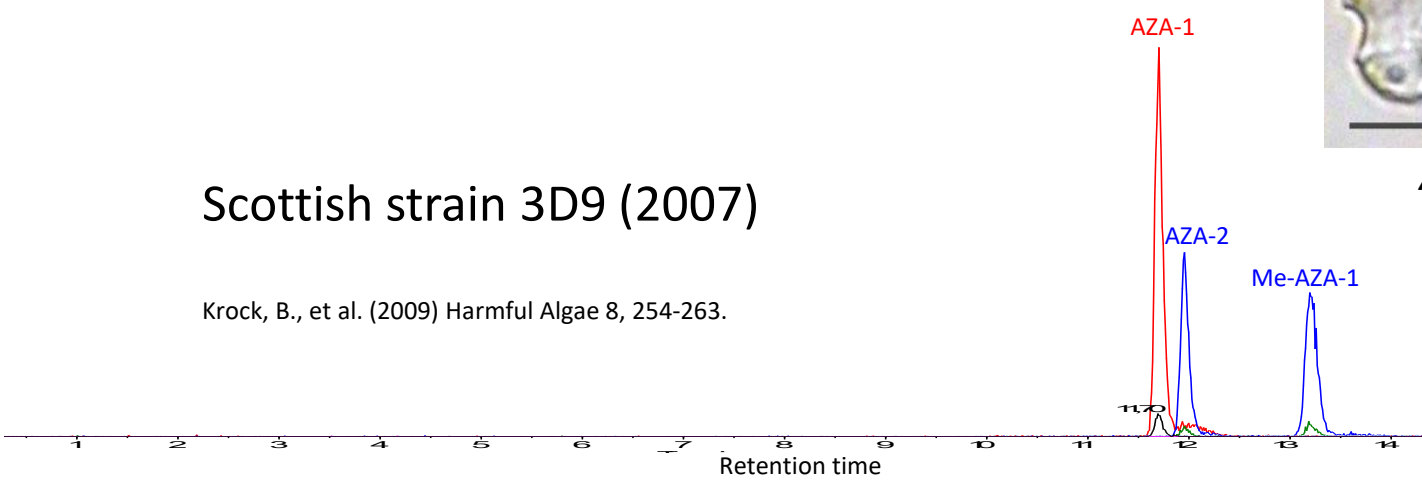
Azadinium as AZA producer



A. spinosum

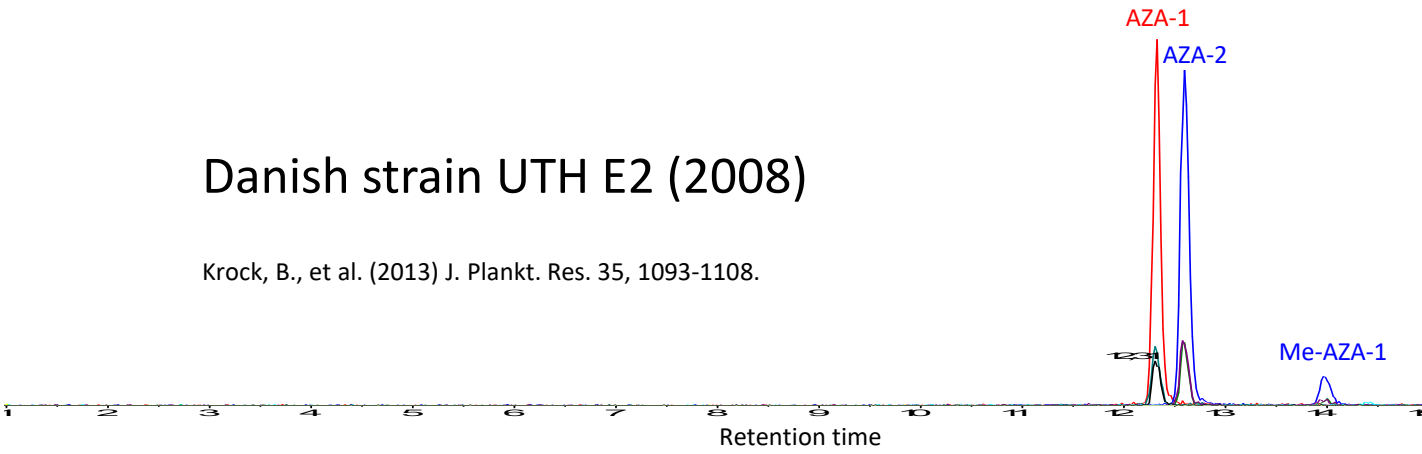
Scottish strain 3D9 (2007)

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



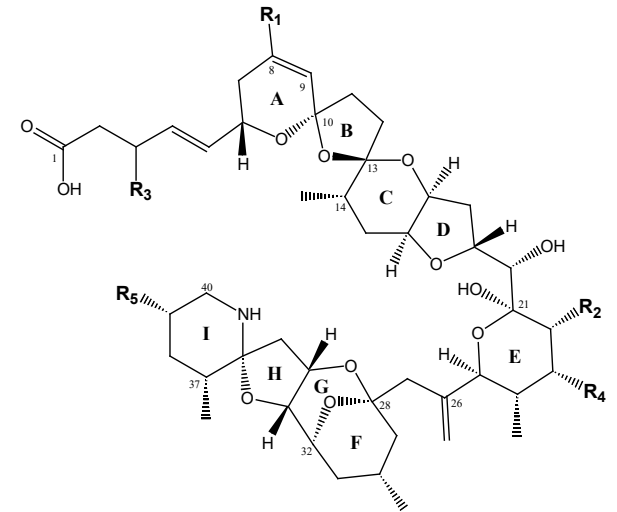
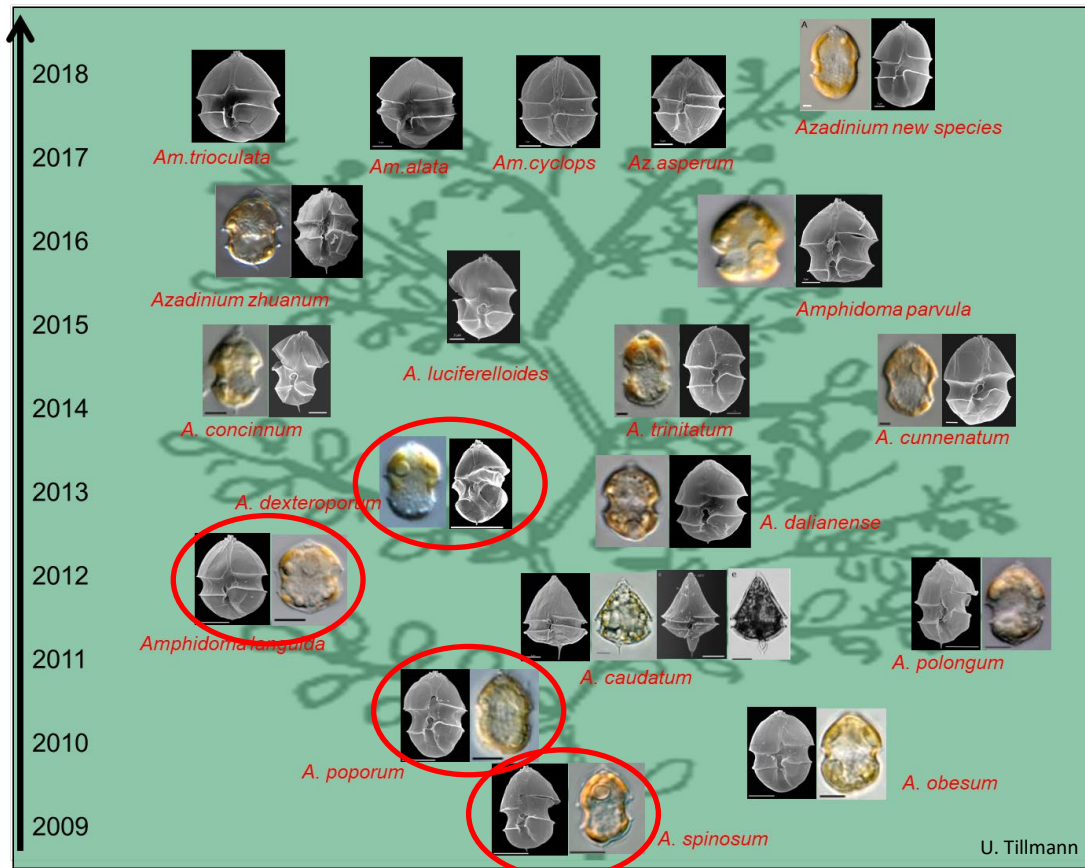
Danish strain UTH E2 (2008)

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

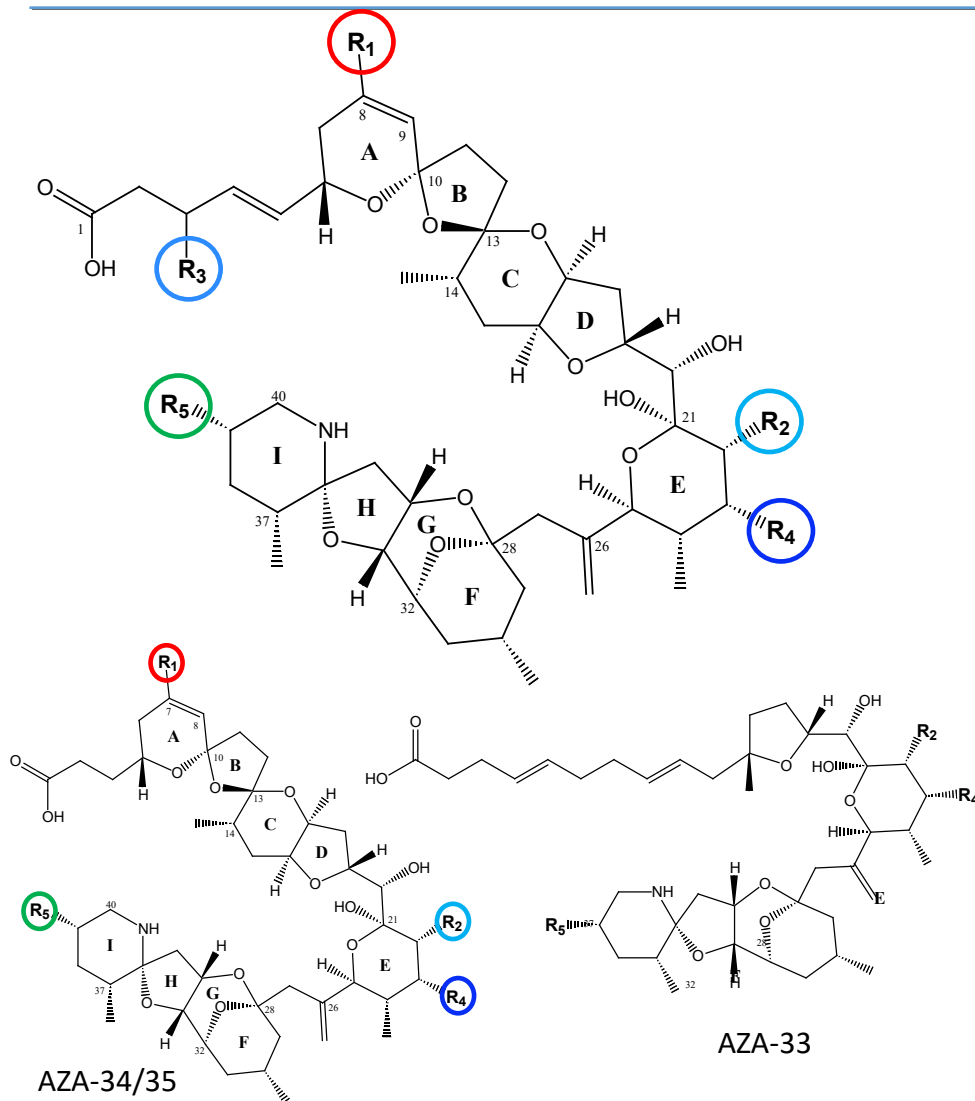


m/z 842>824
 m/z 856>838

5. AZP Toxins



5. AZP Toxins



Toxin	R ₁	R ₂	R ₃	R ₄	R ₅	Δ _{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

5. AZP Toxins



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	phyco toxin	Rehmann et al. 2008
AZA2	H	CH3	H	CH3	H	CH3	CH3	856,5	362 262	spin/pop/lang	phyco toxin	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							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	CH3	CH3	888,5	362 262	shellfish	metabolite	Rehmann et al. 2008
AZA22												Rehmann et al. 2008
AZA23	OH	CH3	H	COOH	H	CH3	CH3	902,5	362 262	shellfish	metabolite	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	CH3	CH3	816,5	362 262	A. spinosum	phyco toxin	Kilkoyne et al. 2014
AZA35	-	CH3	H	CH3	H	CH3	CH3	830,5	362 262	A. spin/A. dexterop	phyco toxin	Kilkoyne et al. 2014, Rossi et al. 2017
AZA36	OH	CH3	H	CH3	H	H	CH3	858,5	348 248	A. poporum	phyco toxin	Krock et al. 2015
AZA37	OH	H	H	CH3	H	H	CH3	846,5	348 248	A. poporum	phyco toxin	Krock et al. 2015
AZA38	nd	nd	nd	nd	nd	nd	nd	830,5	348 248	A. languids	phyco toxin	Krock et al. 2012
AZA39	nd	nd	nd	nd	nd	nd	nd	816,5	348 248	A. languids	phyco toxin	Krock et al. 2012
AZA40	H	CH3	H	CH3	H							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	nd	nd	870,5	362 262	A. dexteroporum	Phycotoxin	Rossi et al. 2017
AZA55	nd	nd	nd	nd	nd	nd	nd	868,5	362 262	A. dexteroporum	Phycotoxin	Rossi et al. 2017
AZA56	nd	nd	nd	nd	nd	nd	nd	884,5	362 262	A. dexteroporum	Phycotoxin	Rossi et al. 2017
AZA57	nd	nd	nd	nd	nd	nd	nd	826,5	362 262	A. dexteroporum	Phycotoxin	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
62 published AZAs

26 AZAs from
planktonic origin

And at least
10 known AZAs

5. AZP Toxins

Azaspiracids - Variants

AZAs produced by algae

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

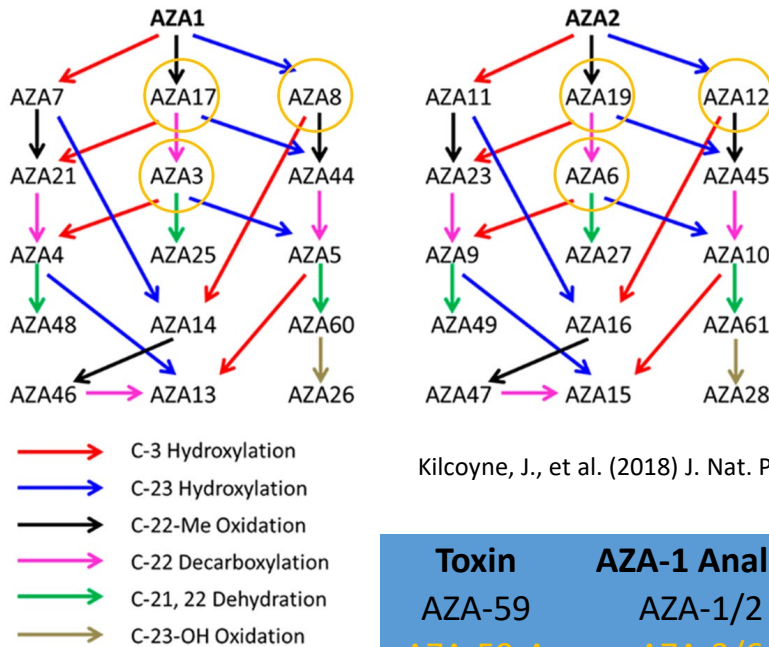
AZA-3	AZA-14	AZA-25	AZA-47
AZA-4	AZA-15	AZA-26	AZA-48
AZA-5	AZA-16	AZA-27	AZA-49
AZA-6	AZA-17	AZA-28	AZA-60
AZA-7	AZA-18	AZA-29	AZA-61
AZA-8	AZA-19	AZA-30	
AZA-9	AZA-20	AZA-31	
AZA-10	AZA-21	AZA-32	
AZA-11	AZA-22	AZA-44	
AZA-12	AZA-23	AZA-45	
AZA-13	AZA-24	AZA-46	

Two AZAs of phytoplankton origin result in 38 shellfish metabolites!

Krock, B., et al. (2019) Harmful Algae 82, 1-8.

5. AZP Toxins

Azaspiracids – Metabolism in Bivalves



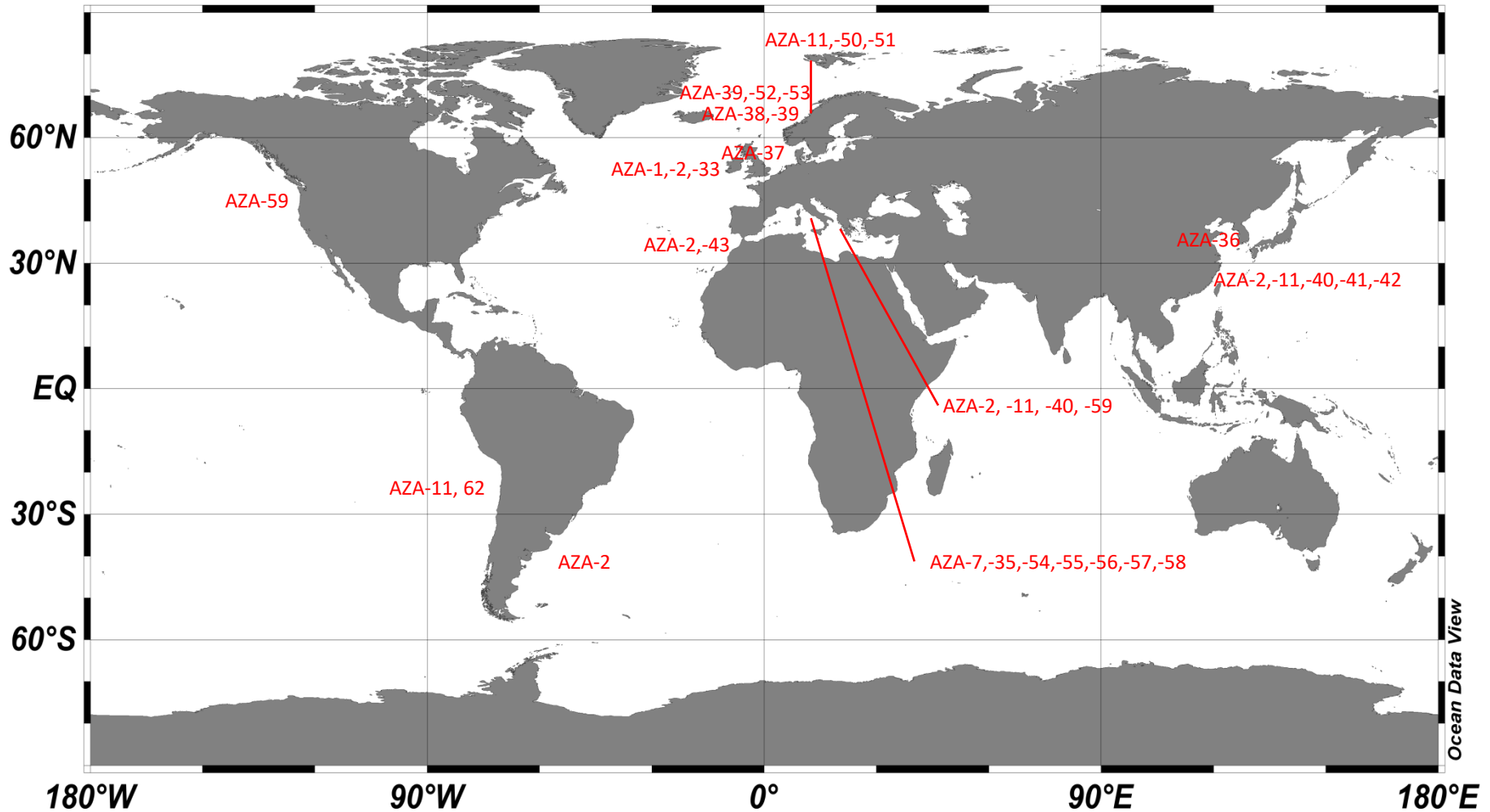
Kilcoyne, J., et al. (2018) J. Nat. Prod. 81(4), 885-893.

Toxin	AZA-1 Analog	m/z [M+H] ⁺	m/z [M+H-H ₂ O] ⁺	Retentiontime [min]
AZA-59	AZA-1/2	860	842	10,6
AZA-59-A	AZA-3/6	846	828	10,1
AZA-59-B	AZA-8/12	876	858	9,6
AZA-59-C	AZA-17/19	890	872	9,9
AZA-59-D	?	890	872	10,3
AZA-59-E	No analog	892	874	9,0
AZA-59-F	No analog	878	860	9,65

Krock, B., et al. in preparation

5. AZP Toxins

Azaspiracids – Geographic distribution

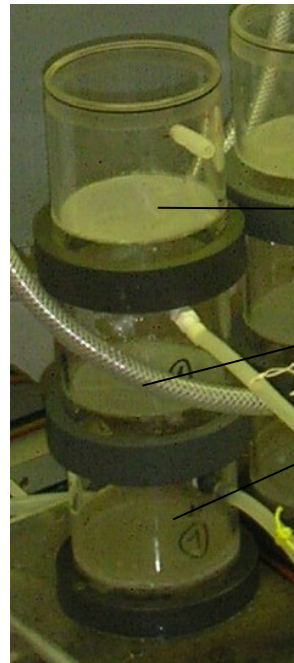


6. Discrete vs. Integrated Monitoring

Phytoplankton Sampling (Discrete Monitoring)



Plankton net
Pore size 20 μm



Filter array



LC-MS/MS

200 μm (zooplankton)

50 μm (big phytoplankton)

20 μm (small phytoplankton)

6. Discrete vs. Integrated Monitoring

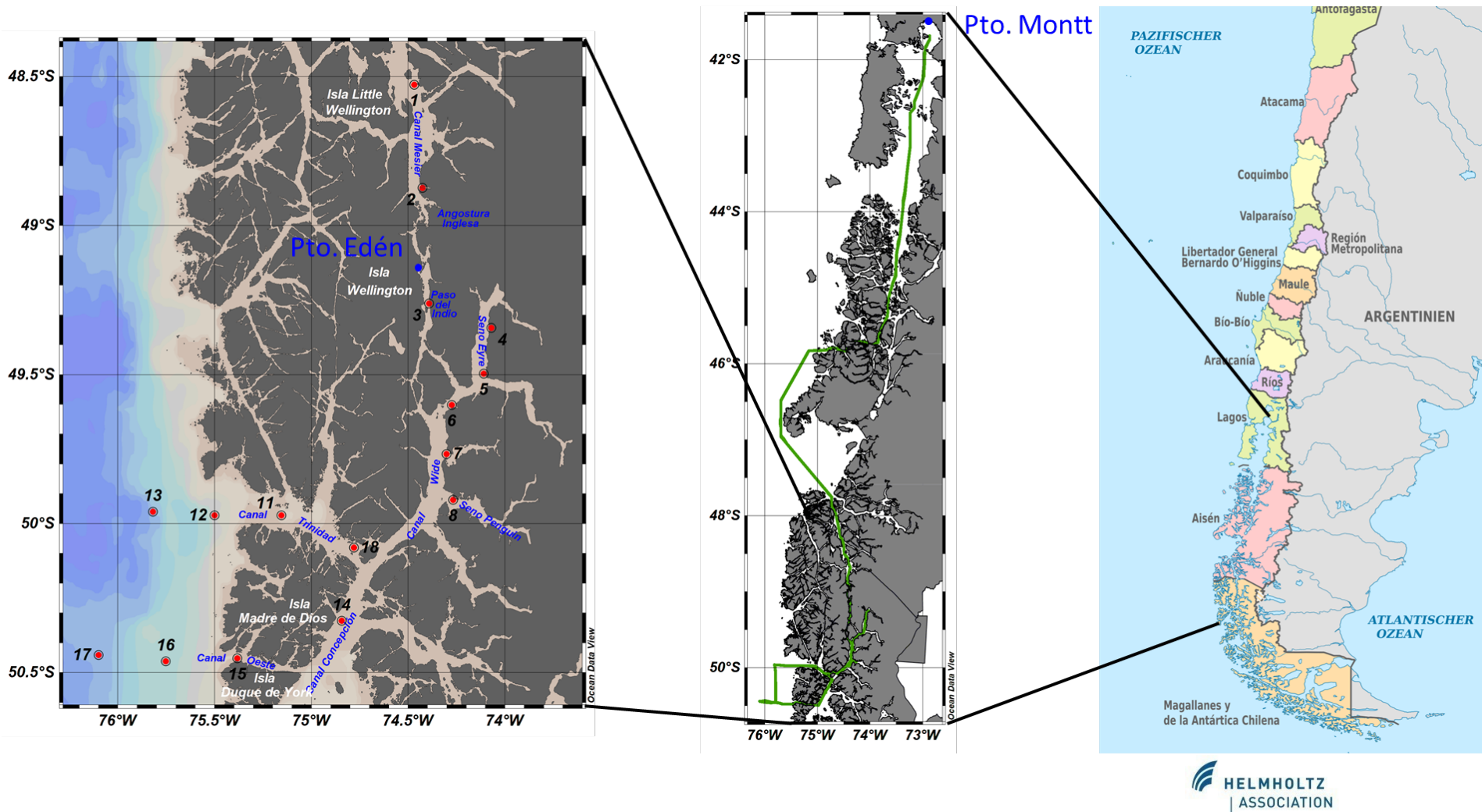
SPATT = Solid Phase Adsorption Toxin Tracking (Integrated Monitoring)

- Water is pumped through a porous lipophilic resin (HP 20)
→ passively adsorbing dissolved toxins in the seawater
- Pros:
 - Time-integrating sampling
→ Toxin dynamics
 - Low-cost; easy sampling
→ Potential as an early warning system
- Cons:
 - Lack of calibration
 - Monitoring of dissolved toxins only
→ no information on the current plankton presence



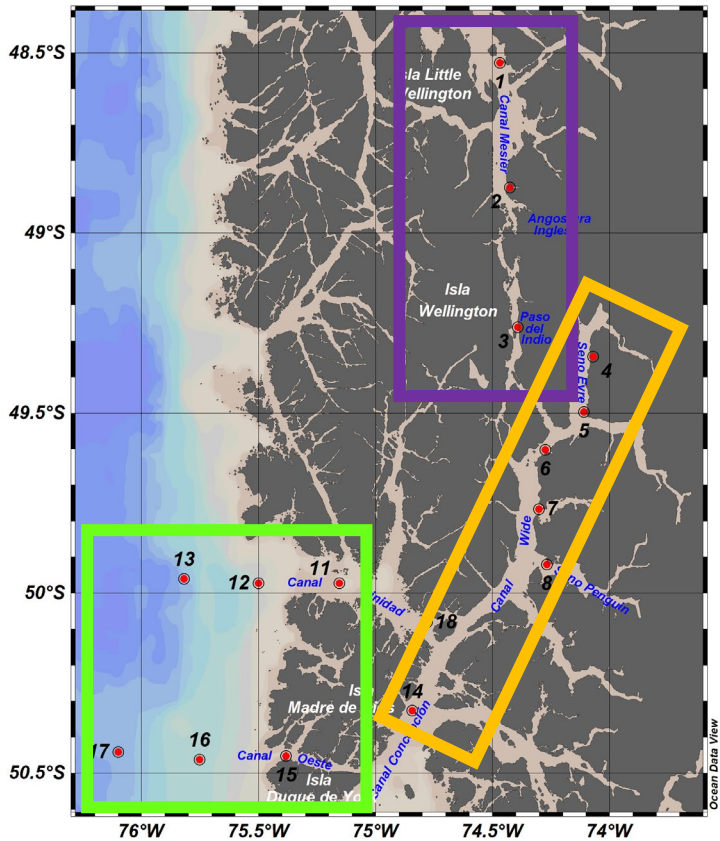
6. Discrete vs. Integrated Monitoring

PROFAN Expedition - Nov. 2019 - RV Cabo de Hornos

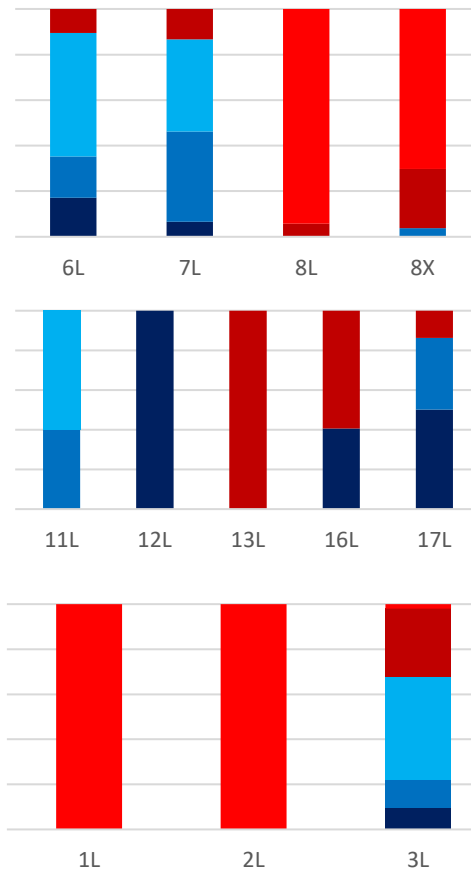


6. Discrete vs. Integrated Monitoring

■ OA
 ■ DTX1
 ■ YTX
 ■ homo-YTX
 ■ 45 OH Homo YTXs
 ■ PTX2
 ■ PTX2sa



Discrete sampling
(Stations)

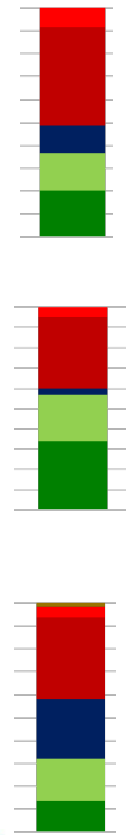


Integrated sampling
(SPATTs)

Area 2

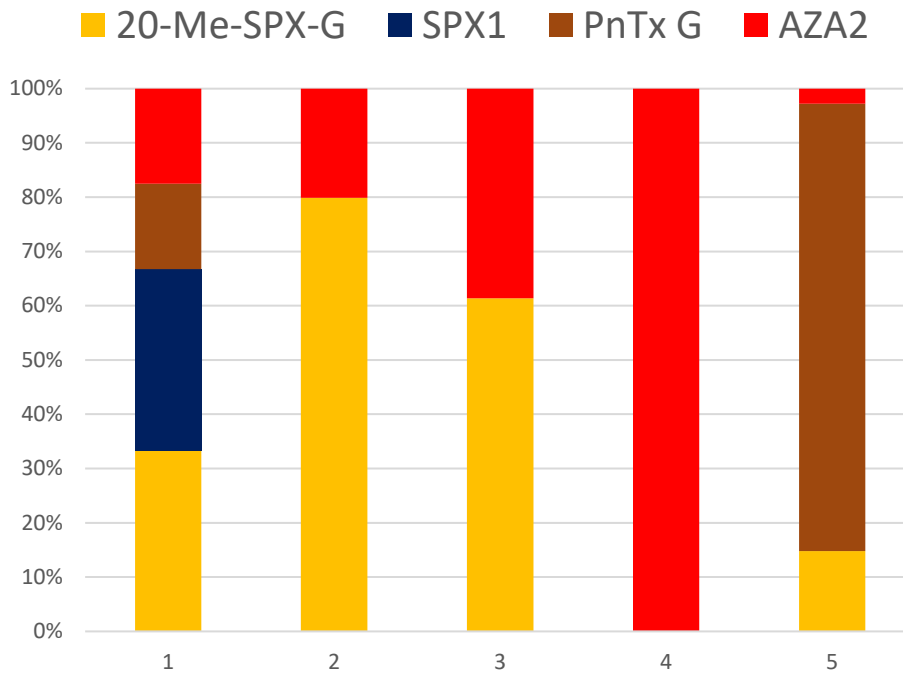
Area 3

Area 4



6. Discrete vs. Integrated Monitoring

Integrated sampling (SPATTs)



20-Me-SPX-G, SPX1
(spiroolides)

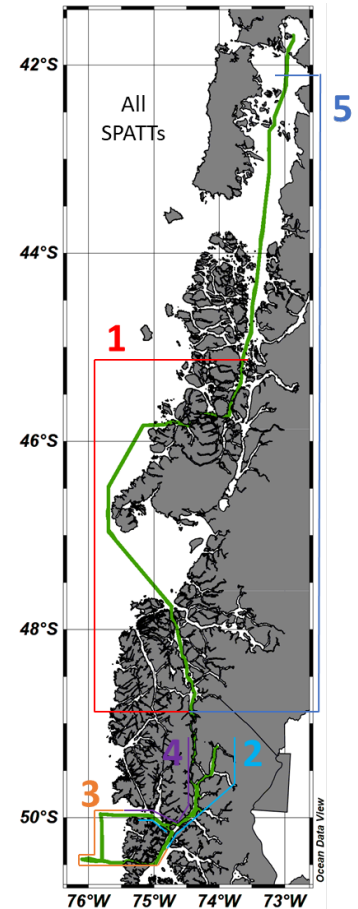
Alexandrium ostenfeldii

PnTx G (pinnatoxins)

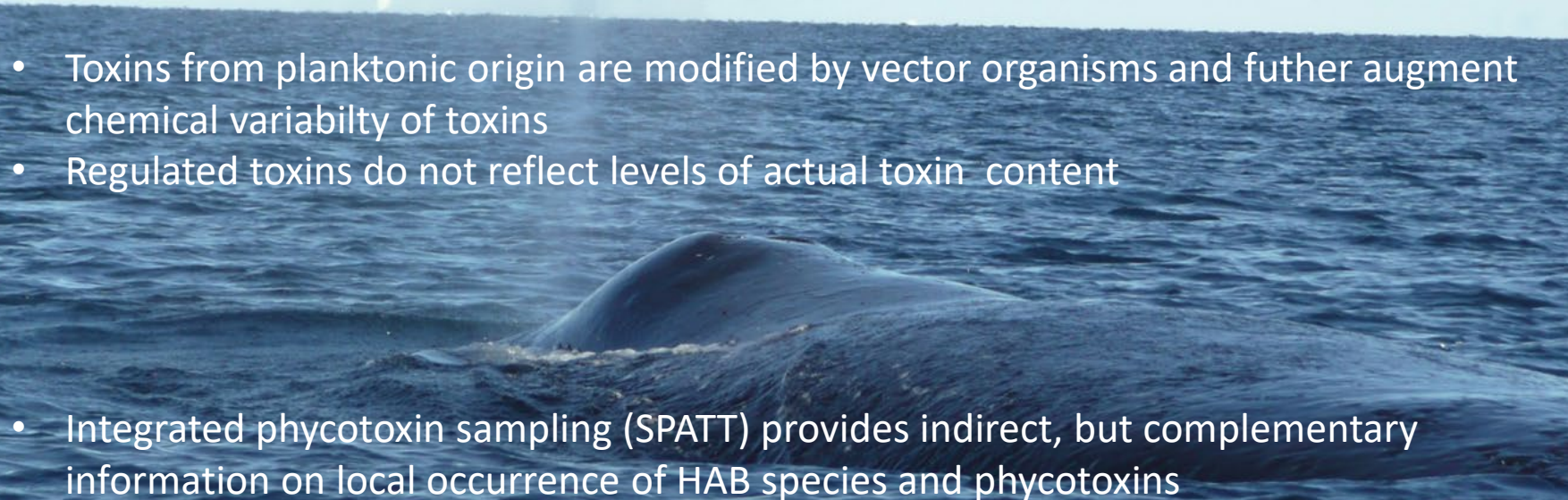
Vulcanodinium rugosum

AZA2 (azaspiracids)

Azadinium poporum



Conclusions

- Phycotoxin variability of known toxin classes is high and yet not fully explored
 - Phycotoxin profiles are geographically very variable
 - Phycotoxin profiles in a given area may change over time
 - Ichthyotoxins are rarely characterized, but pose an increasing threat due to increasing aquacultural activities
- 
- Toxins from planktonic origin are modified by vector organisms and further augment chemical variability of toxins
 - Regulated toxins do not reflect levels of actual toxin content
- Integrated phycotoxin sampling (SPATT) provides indirect, but complementary information on local occurrence of HAB species and phycotoxins

Thanks for
Your Attention!





Scientific FjordFlux Crew in front of Cape Horn