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HABs and phycotoxins: a general view and impacts on Chile

Bernd Krock

Alfred Wegener Institut-Helmholtz Zentrum für Polar- und Meeresforschung
Am Handelshafen 12. 27576 Bremerhaven, Germany

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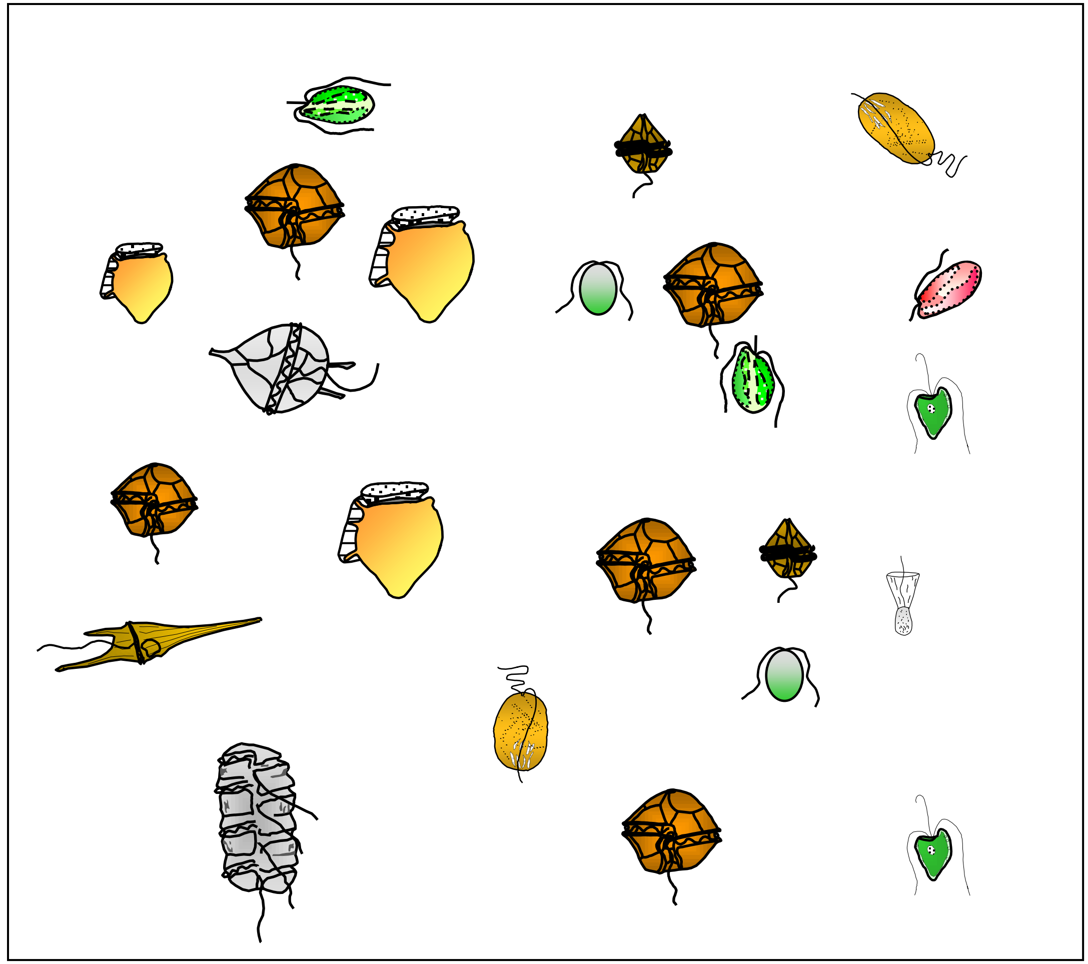
Hab Events

Two aspects:

1. **Shellfish poisoning syndromes**

2. **Ichthyotoxic (fishkilling) HAB Events**



Hab Events

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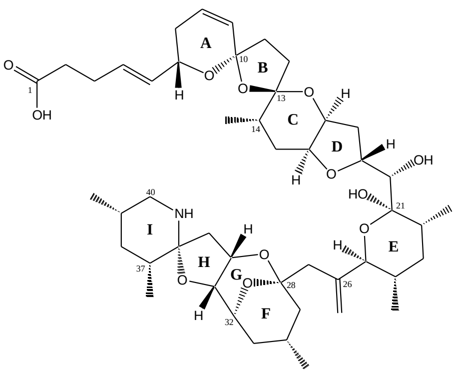
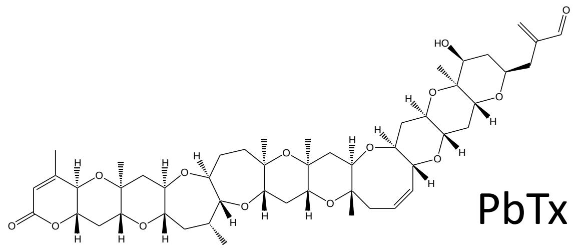
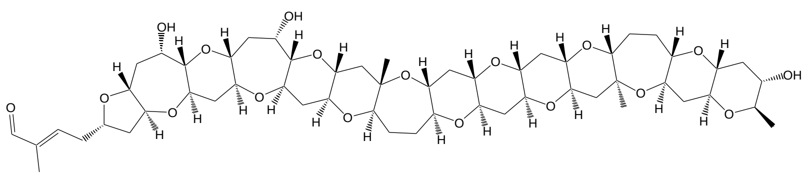


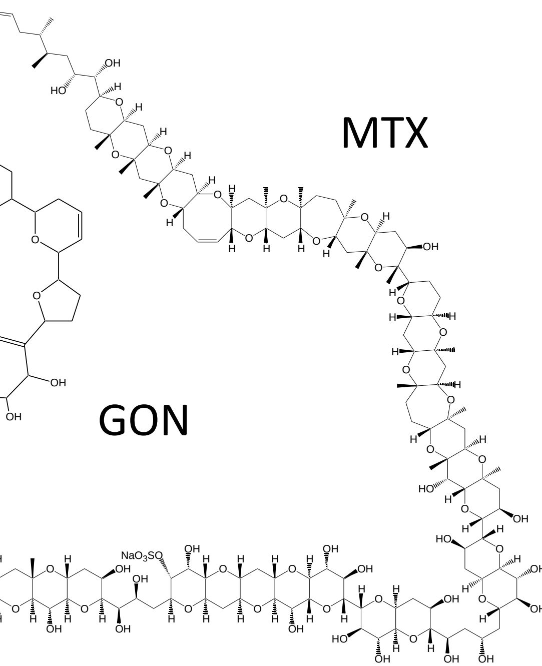
AZA



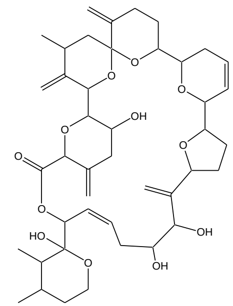
PbTx



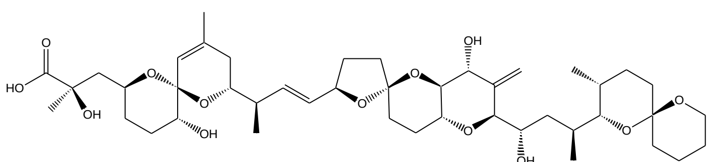
CTX



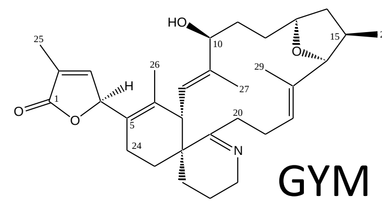
MTX



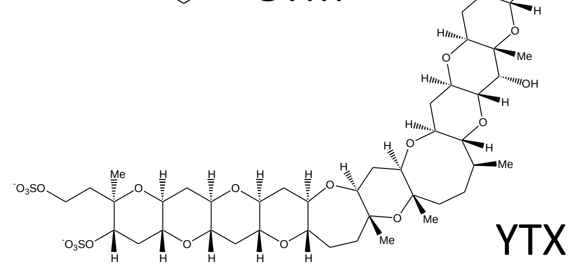
GON



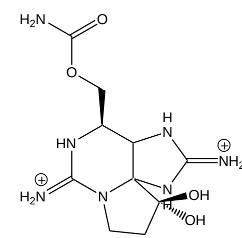
DTX



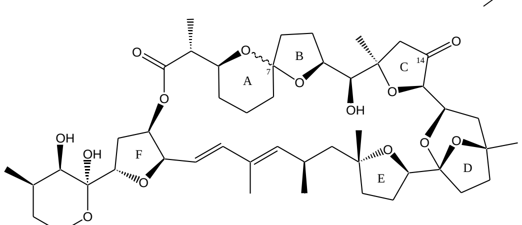
GYM



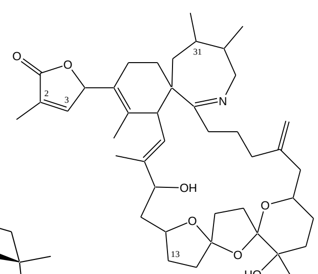
YTX



PST



PTX



SPX

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Hab Events

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Toxic effects on vertebrates (incl. humans):

Amnesic shellfish poisoning	**ASP**
Paralytic shellfish poisoning	**PSP**
Diarrhetic shellfish poisoning	**DSP**
Neutoxic shellfish poisoning	**NSP**
Spiroimine shellfish poisoning	**SSP**
Ciguatera fish poisoning	**CFP**
Azaspiracid shellfish poisoning	**AZP**

Why select SSP and AZP?

- **Belong to the least researched syndromes**
- **Have an enormous toxin variability**
- **High potential of future risks**

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SSP

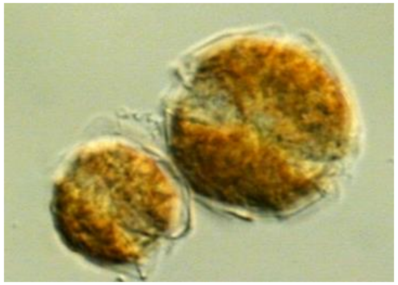
Spiroimines

Spirolide toxicity:

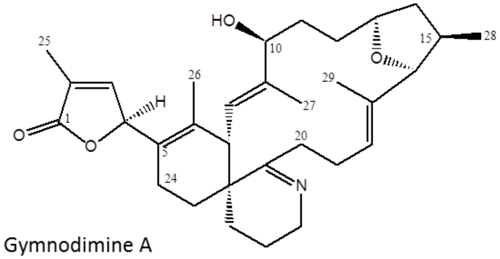
LD$_{50}$ (ip) in mice: 40 µg/kg
Oral toxicity: 1 mg/kg

Gymnodimine toxicity:

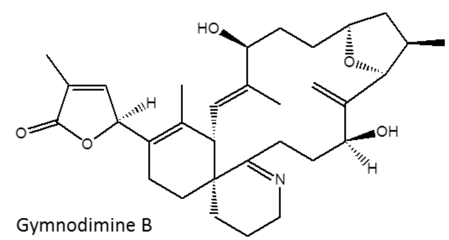
LD$_{50}$ (ip) in mice: 96µg/kg



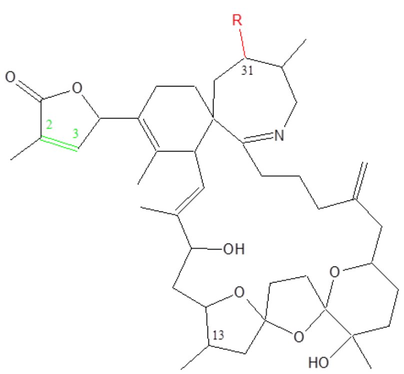
Alexandrium ostenfeldii



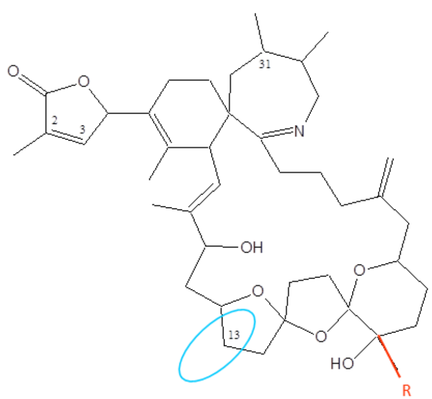
Gymnodimine A



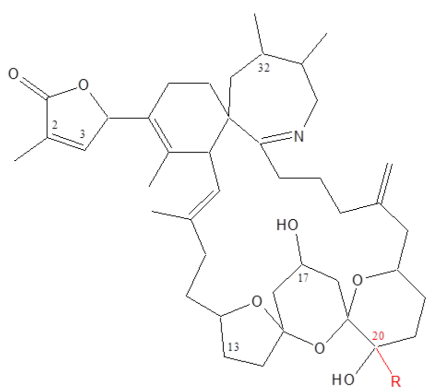
Gymnodimine B



Spirolide A: R = H, Δ^{2,3}
B: R = H
C: R = Me, Δ^{2,3}
D: R = Me



13-DesMe Spirolide C: R = Me
13,19-DidesMe Spirolide C: R = H



Spirolide G: R = H
20-Me Spirolide G: R = Me

SSP

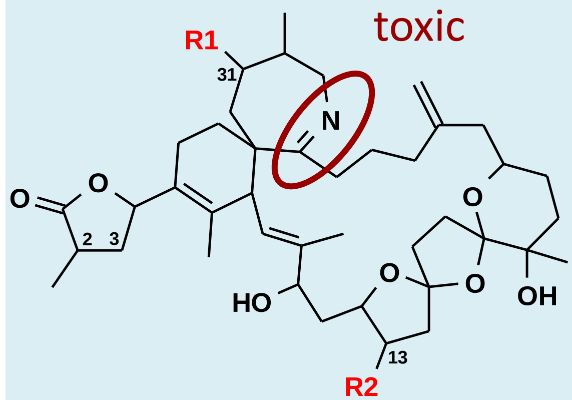
Spirolides:

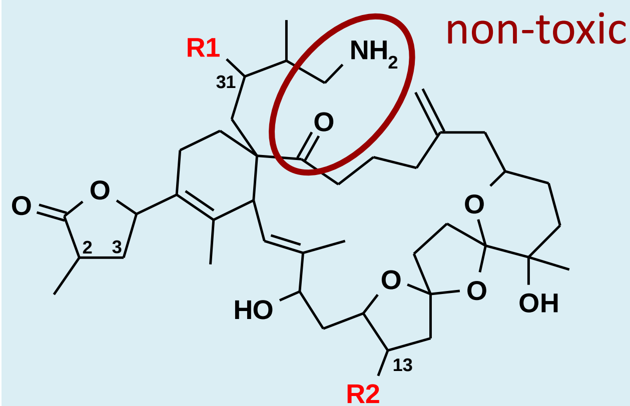
- macrocyclic imines
- structural similarity to pinnatoxins & gymnodimines
- pharmacologically active/inactive forms

Mode of Action:

anticholinergic activity by blocking of muscarinic acetylcholin receptors

=> Paralysis of the parasympathetic nervous system (few toxicological studies)





	R1	*R2*		*MW*
A	H	CH_3	$\Delta^{2,3}$	691.5
desMeC	CH_3	H	$\Delta^{2,3}$	691.5
B	H	CH_3		693.5
desMeD	CH_3	H		693.5
C	CH_3	CH_3	$\Delta^{2,3}$	705.5
D	CH_3	CH_3		707.5
E	H	CH_3	$\Delta^{2,3}$	709.5
F	H	CH_3		711.5

SSP high toxin variabilty ***within*** one strain

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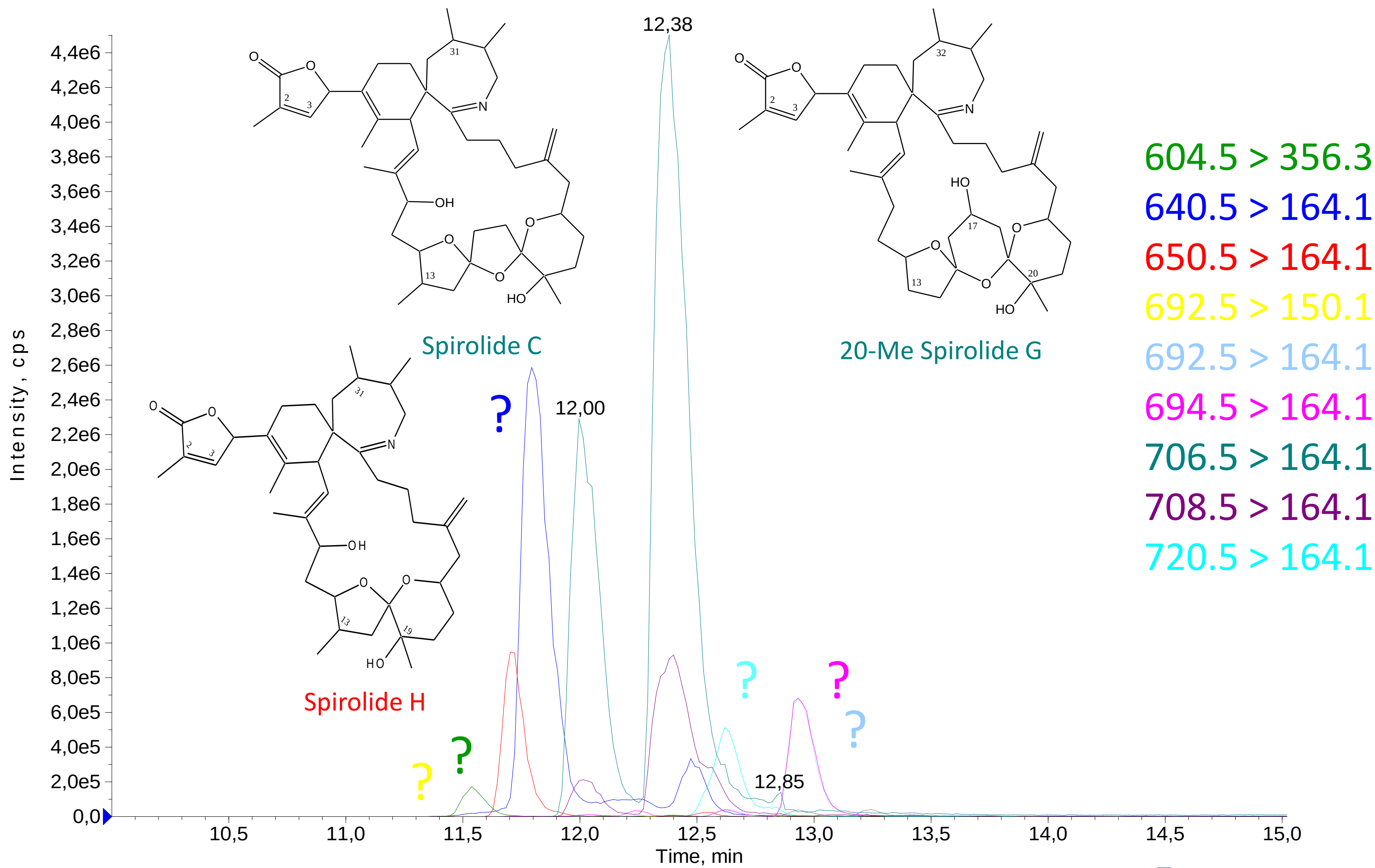
Alexandrium ostenfeldii (Ship Harbor, NS, Canada, AOSH2)



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SSP high toxin variabilty ***within*** populations

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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		0.3	-	0.8		-	**13.7**
P2 E3	516	**31.2**	-	-	**41.3**	-	**27.5**	-	-		-	-	-
P2 E4	516	**19.2**	-	-	-	1.2	-	-	-	**70.1**	-	-	**9.4**
P2 F2	516	**5.1**	**63.6**	-	**6.7**	0.1	**4.1**	-	-	**10.6**	-	**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**	-	-	-	0.3	-	-	-	-	-
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	-

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

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SSP high toxin variabilty *within* populations

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known variants

Mass transition	toxin
508>490	GYM A
522>504	12-me GYM A
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, undescribed, pinnatoxin G
694>180	27-hydroxy-13,19-didesme C
694>150	B
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 et al. (2014) Harmful Algae 39, 259-270.

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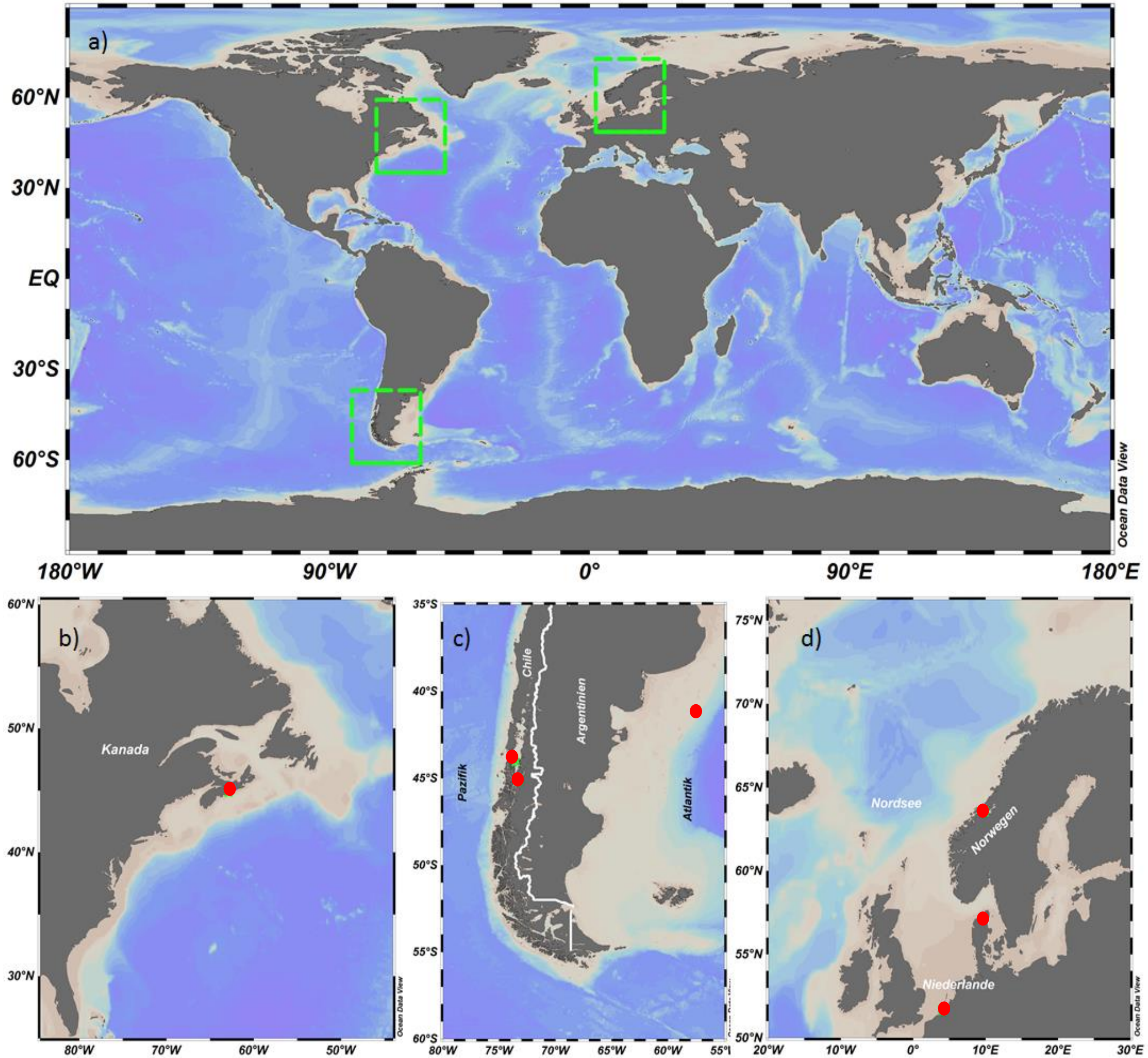

Krohn 2016 (2012) Bachelor thesis, University of Lübeck, Germany

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SSP high toxin variabilty *among* populations

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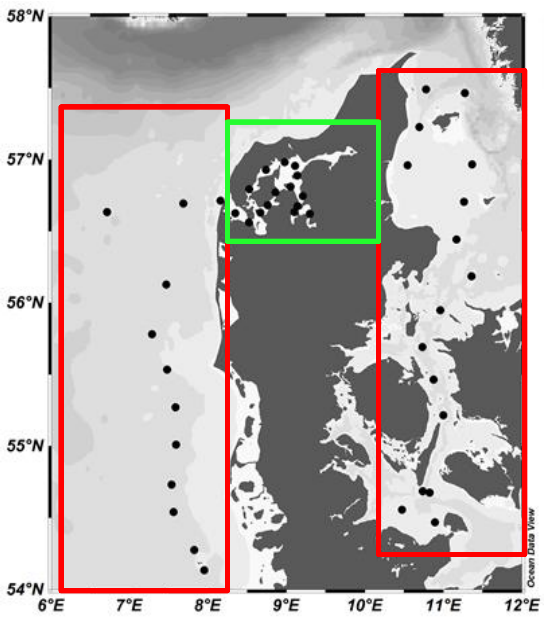
Strain	Salinity	Origin	PSP	SPX	GYM
AOSH2	> 30	Atlantic Canada	/	x	/
NX 56-07	21,1 - 33,3	Norway	/	x	x
NX 56-10	21,1 - 33,4	Norway	/	x	x
NX 56-12	21,1 - 33,5	Norway	/	x	x
ND	33,3	North Sea*	/	x	/
ND	26,1	Limfjord DK*	/	x	x
ND	24,5	Kattegat*	/	x	/
OKNL 42	8 - 21	The Netherlands	x	x	x
AOICW	32,5	Chile	x	x	/
AOA32-2	28,2	Chile	x	x	/
H1G8	33,6	Argentina	x	x	/
H3D4	33,6	Argentina	x	x	/
H2-A4	33,6	Argentina	x	x	/

* Field samples



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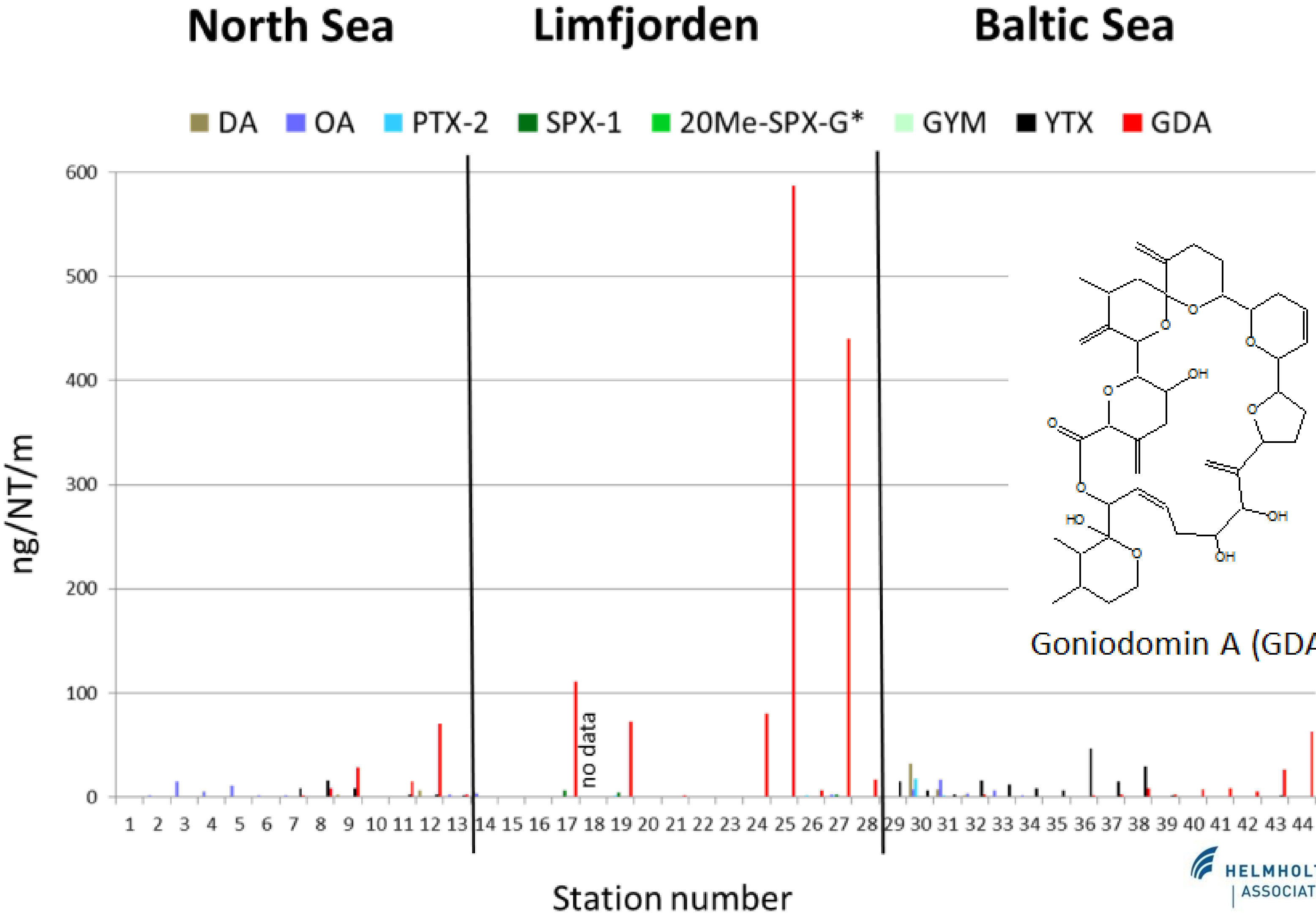
Krohn 2016 (2012) Bachelor thesis, University of Lübeck, Germany

Dinoflagellate community shift



Goniodomin A (GDA)

Dinoflagellate community shift

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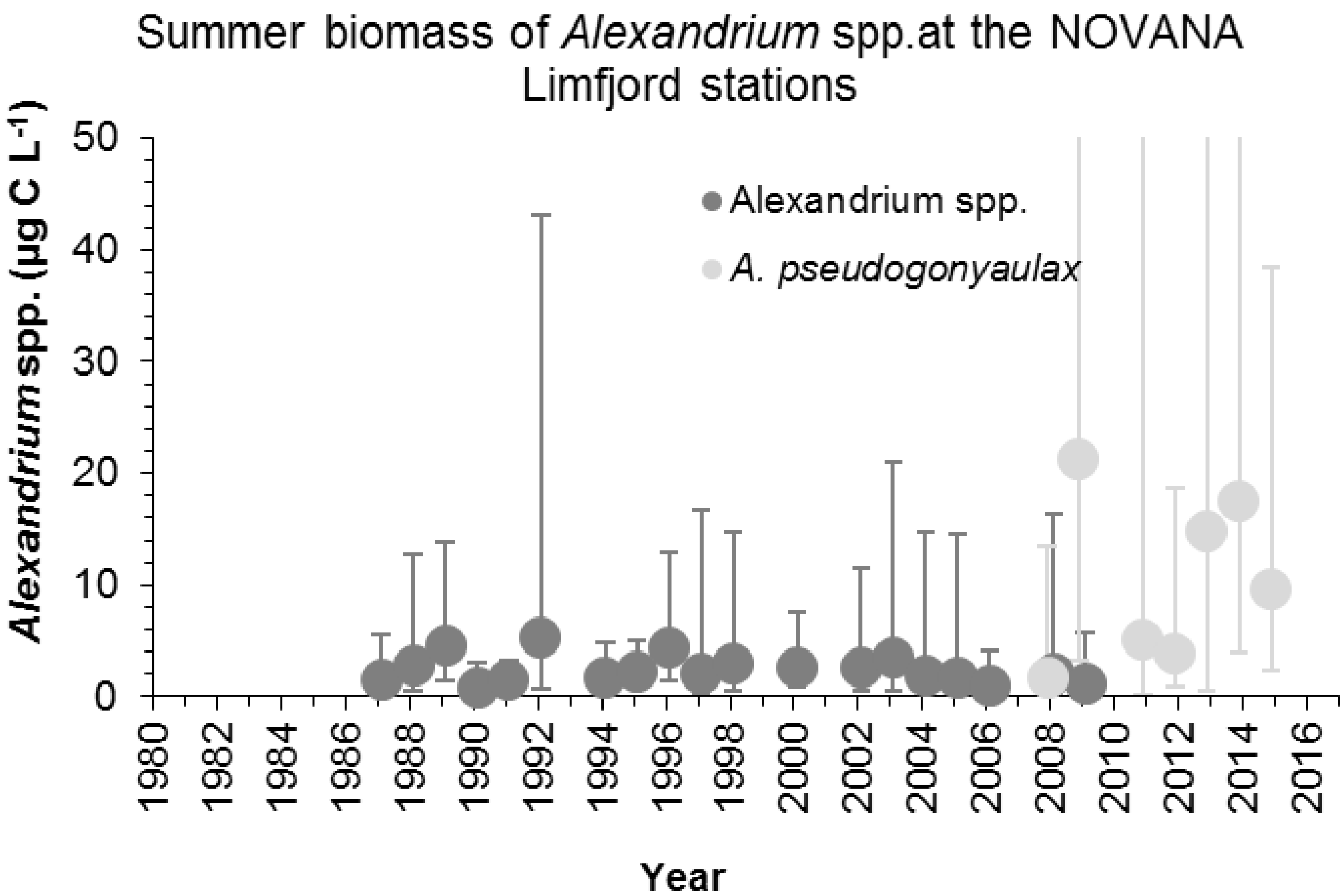

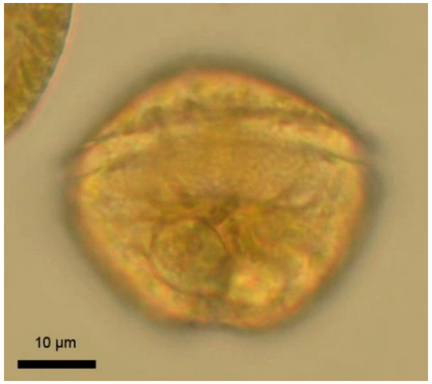

Alexandrium pseudogonyaulax

Producer of Goniodomins

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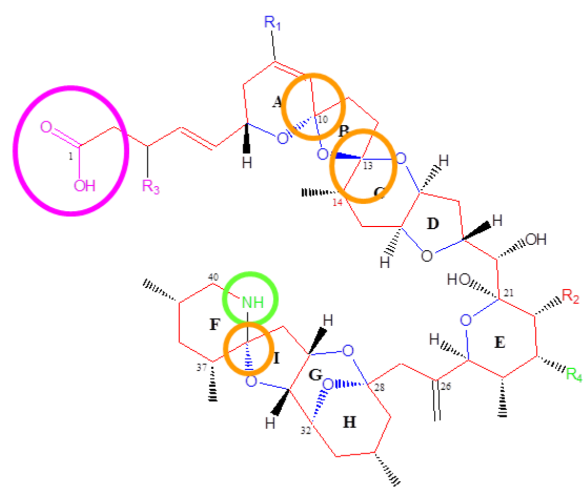
AZP

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 (MacMahon & Silke, 1996: Harmful Algae News, 14, 2)

Toxicity:

LD$_{50}$ AZA-1: 0.2 µg/kg (mice)



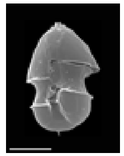
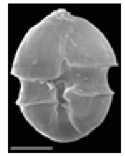
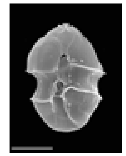
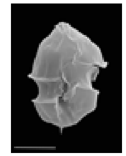
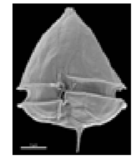
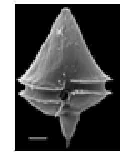
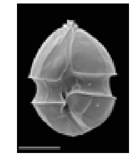
azaspiracid

Polyketide:
linear carbon skeleton
with cyclic ether bridges
amino function
chemical nomenclature:
aza = secondary amine
spiro function
acid

Mode of Action:

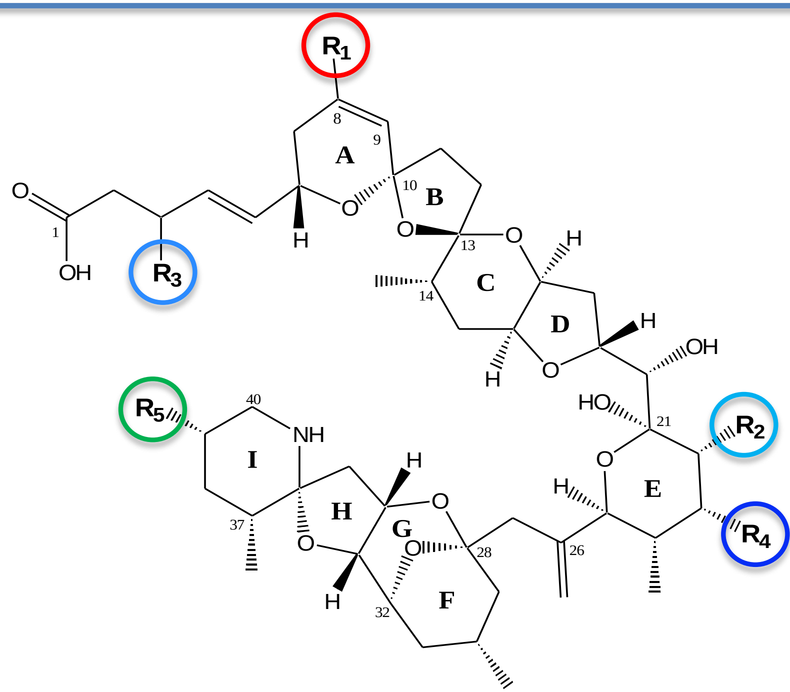
- effects of AZA-1 on the arrangement of F-actin
  ⇒concurrent loss of pseudopodia, cytoplasmic extensions that function in mobility and chemotaxis; effects on cytoskeleton
- Increases cytosolic calcium levels in lymphocytes
- diarrheagenic, tumorigenic

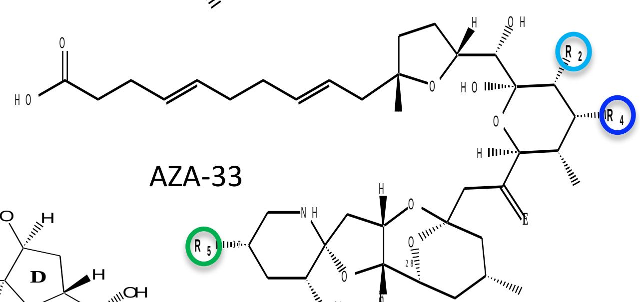
AZP

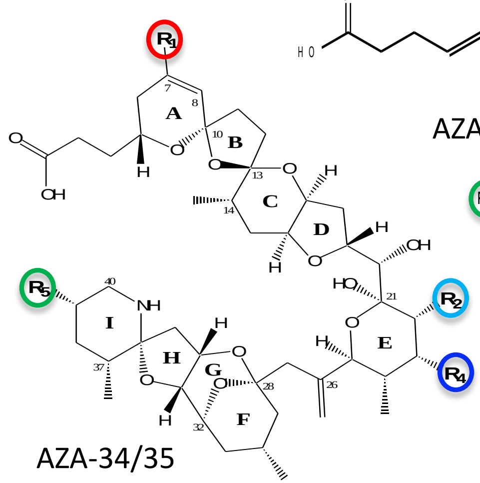








	Azadinium spinosum	*Azadinium obesum*	*Azadinium poporum*	*Azadinium polongum*	*A. caudatum* var. *margalefii*	*A. caudatum* var. *caudatum*	*Amphidoma languida*
size	13.8 x 8.8	15.3 x 11.7	13.0 x 9.8	13.0 x 9.7	31.1 x 22.4	41.7 x 28.7	13.9. x 11.9
length/width ratio	1.6	1.3	1.3	1.3	1.2	1.2	1.2
Pyrenoid	1; epicone	-	Several (4?), epi- and hypocone	-	-	-	1; epicone
Spine	+	-	-	+	**Short horn, long spine**	**Long horn, short spine**	
Ventral pore	+	+	-	+	-	+	+
Pore on po-plate	-	-	+	-	+	-	-
Shape pore-plate	**round-elipsoid**	**round-elipsoid**	**round-elipsoid**	**elongated**	**round-elipsoid**	**round-elipsoid**	**round-elipsoid**
Plate 1´´ in contact to plate 1a	+	-	+	+	+	+	+
Plate 6´´ in contact to plate 3a	-	-	-	-	+	+	-
Azaspiracids	+	-	+	-	-	-	+

AZP







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	nd	nd	nd	nd	H	nd	842
AZA-41	nd	nd	nd	nd	CH_3	nd	854
AZA-42	nd	nd	nd	nd	CH_3	nd	870

AZP

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Planktonic strain AZAs

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 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-7	AZA-18	AZA-29	
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 36 shellfish metabolites!

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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	CH3	CH3	858,5	362 - 262	A. dexteroporum	phycotoxin	Rossi et al. 2017
AZA8	H	H	H	CH3	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA9	OH	CH3	H	H	H	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA10	H	CH3	H	H	OH	CH3	CH3	858,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA11	OH	CH3	H	CH3	H	CH3	CH3	872,5	362 - 262	Pop/shellf	phycotox, metabol	Rehmann et al. 2008
AZA12	H	CH3	H	CH3	OH	CH3	CH3	872,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA13	OH	H	H	H	OH	CH3	CH3	860,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA14	OH	H	H	CH3	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	Rehmann et al. 2008
AZA15	OH	CH3	H	H	OH	CH3	CH3	874,5	362 - 262	shellfish	metabolite	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	CH3	CH3	810,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA26	H	H	H	H	O	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA27	H	CH3	H	H	H	CH3	CH3	824,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA28	H	CH3	H	H	O	CH3	CH3	838,5	362 - 262	shellfish	metabolite	Kilkoyne et al. unpubl.
AZA29	H	H	CH3	H	H	CH3	CH3	842,5	362 - 262	shellfish	artefact	Rehmann et al. 2008
AZA30	H	H	CH3	CH3	H	CH3	CH3	856,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA31												Rehmann et al. 2008
AZA32	H	CH3	CH3	CH3	H	CH3	CH3	870,5	362 - 262	A. spinosum	artefact	Rehmann et al. 2008
AZA33	–	–	H	CH3	H	CH3	CH3	716,5	362 - 262	A. spinosum	phycotoxin	Kilkoyne et al. 2014
AZA34	–	H	H	CH3	H	CH3	CH3	816,5	362 - 262	A. spinosum	phycotoxin	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	CH3	CH3	854,5	360-260	A. poporum	phycotoxin	Krock et al. 2014
AZA42	OH	CH3	H	CH3	H	CH3	CH3	856,5	360-260	A. poporum	phycotoxin	Krock & Tillmann, unpubl.
AZA43	–	H	H	CH3	H	H	CH3	828,5	360-260	A. languida	phycotoxin	Tillmann et al. 2017
AZA44	H	H	H	COOH	OH	CH3	CH3	888,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA45	H	CH3	H	COOH	OH	CH3	CH3	902,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA46	OH	H	H	COOH	OH	CH3	CH3	904,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA47	OH	CH3	H	COOH	OH	CH3	CH3	918,5	362-262	shellfish	metabolite	Kilkoyne et al. 2015
AZA48	OH	H	H	H	H	CH3	CH3	826,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA49	OH	CH3	H	H	H	CH3	CH3	840,5	362-262	shellfish	metabolite	Kilkoyne unpublished
AZA50	H	CH3	H	CH3	H	CH3	H	842,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA51	OH	CH3	H	CH3	H	CH3	H	858,5	348-262	A. spinosum	Phycotoxin	Krock & Tillmann, unpubl.
AZA52	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
AZA53	nd	nd	nd	nd	nd	nd	nd	830,5	348-248	A. languida	Phycotoxin	Krock & Tillmann, unpubl.
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

Currently
60 known AZAs

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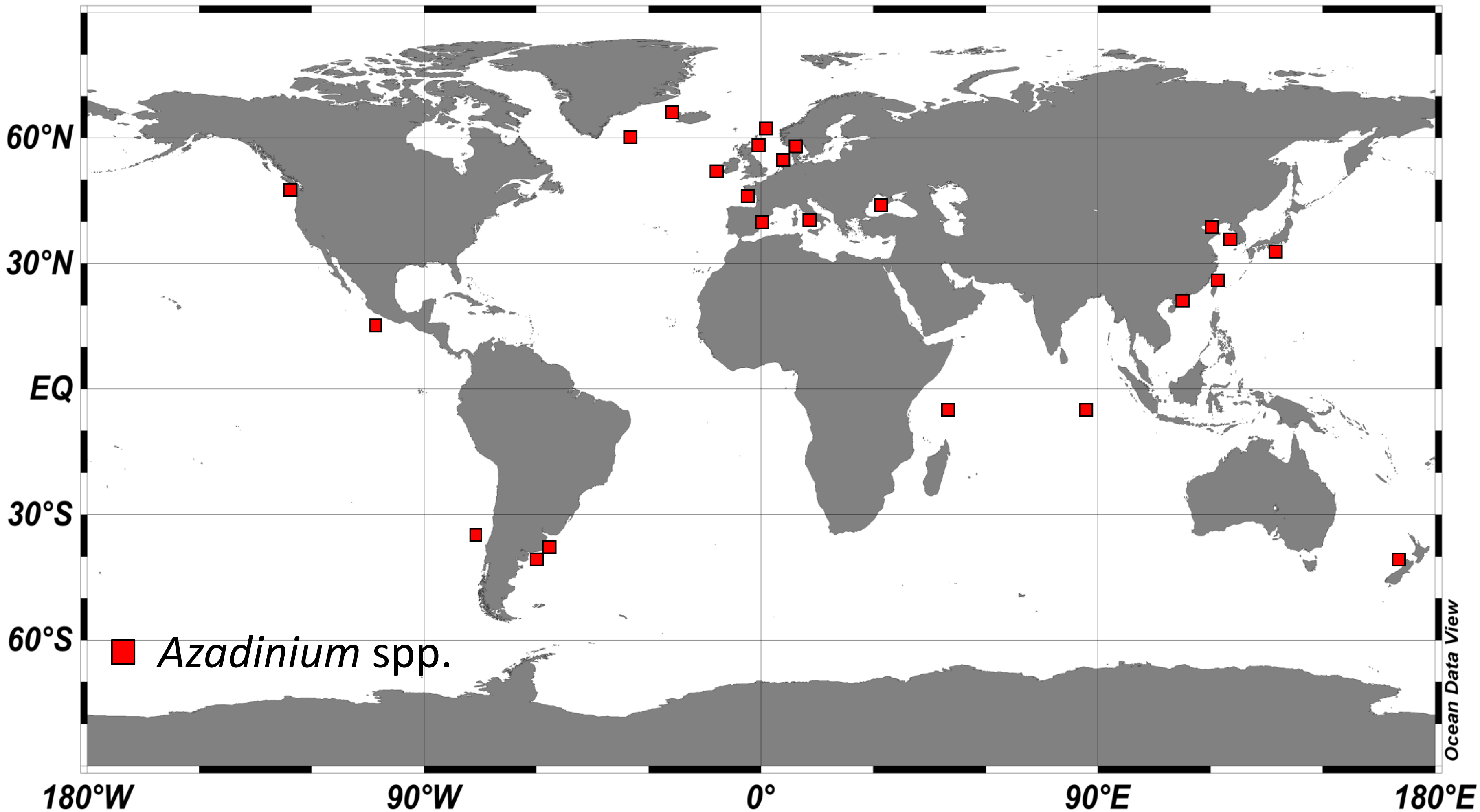
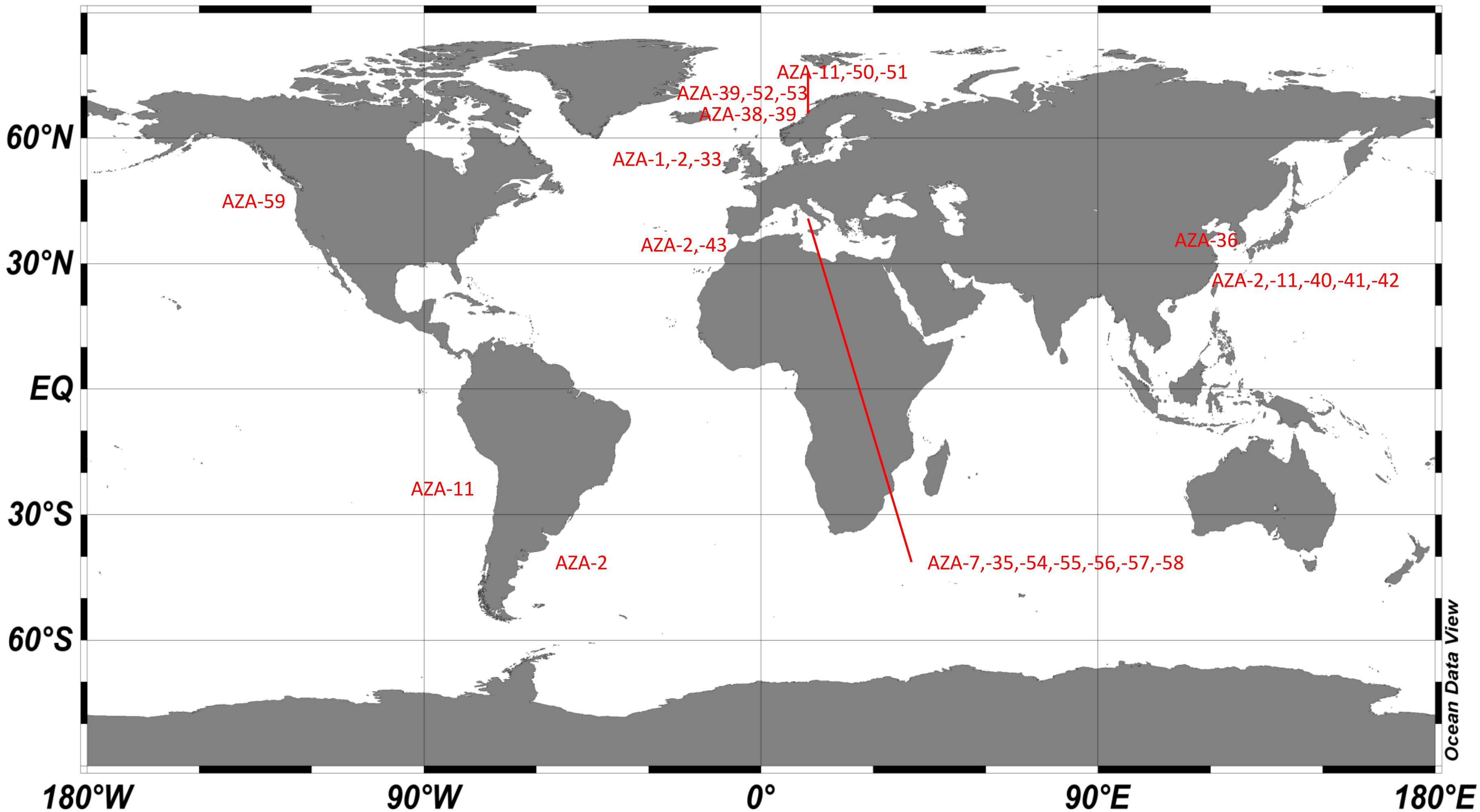
AZP

Occurrence of *Azadinum* spp. & *Amphidoma languida*



AZP

AZA profiles of species and strains of different origins



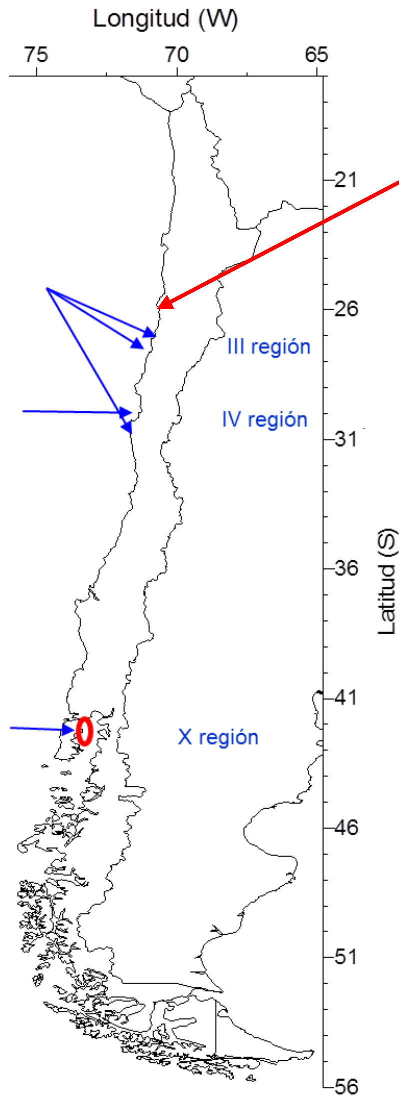
AZP

➢ AZPs en Chile

Azadimium spinosum

Reports of AZA-1, -2 -3 and -6 in mollusks

Pizarro, G. pers. comm.



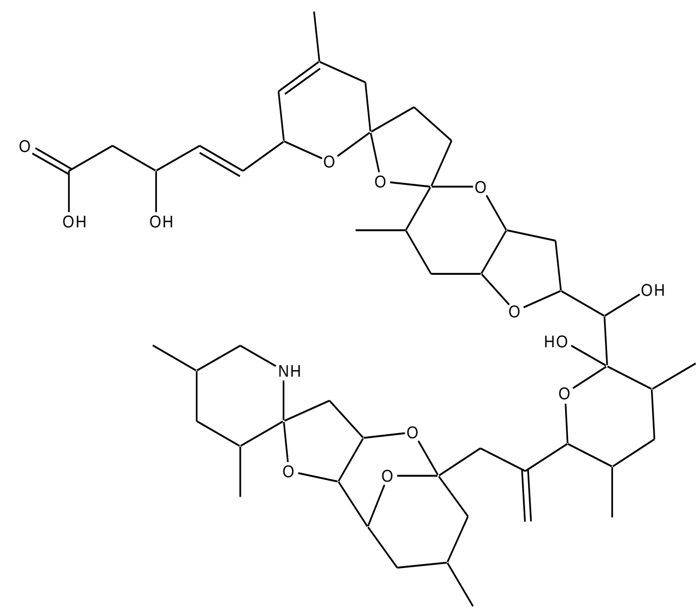
Límite normativo máximo
(consumo humano)
160 µg/kg

Azadinium poporum

Chañaral area

Tillmann et al. (2017) J. Plankt. Res., 39, 350-367.

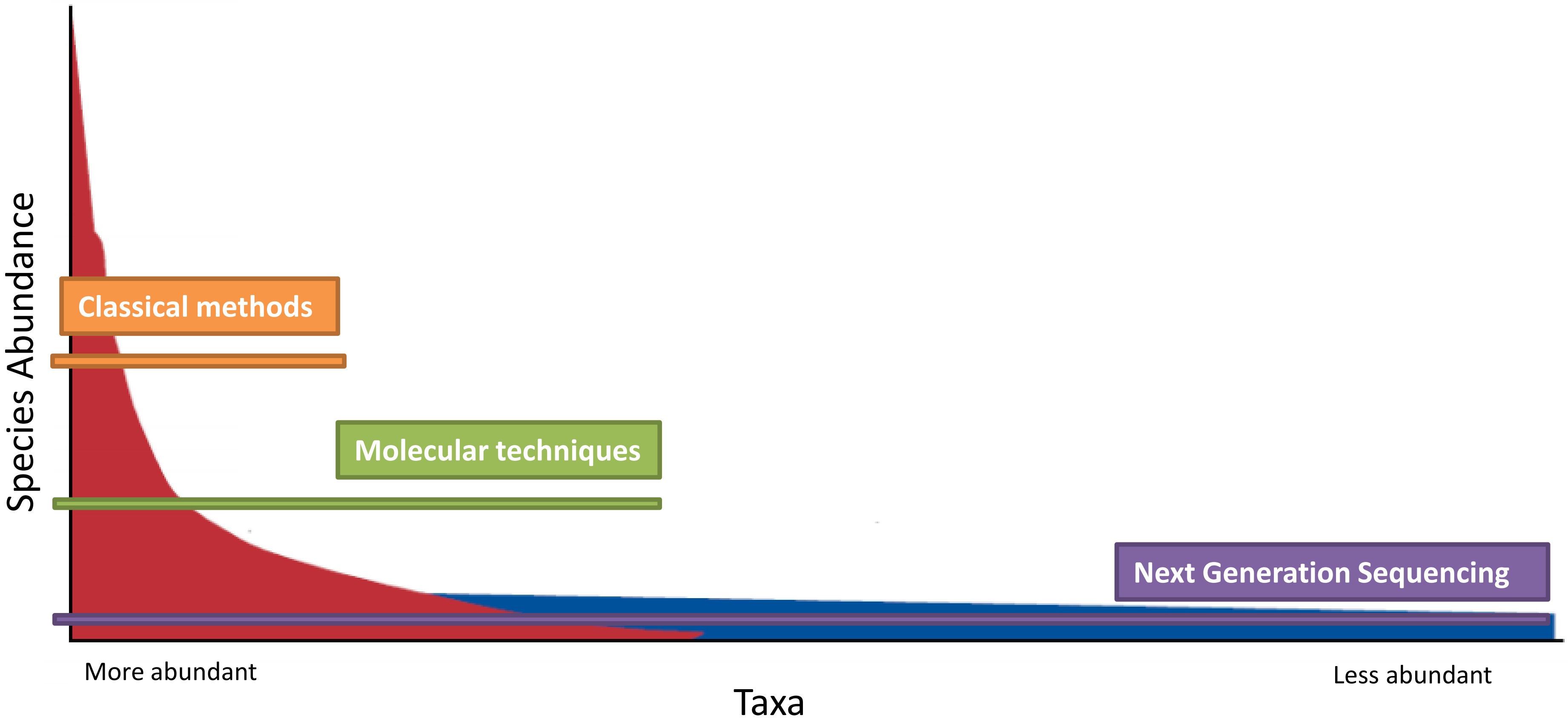
AZA-11 (3-4 fg/cell)
and
AZA-11 phosphate



Cryptic species

Metagenomics of plankton

Moreno-Pino et al., submitted



HAB species range expansion?

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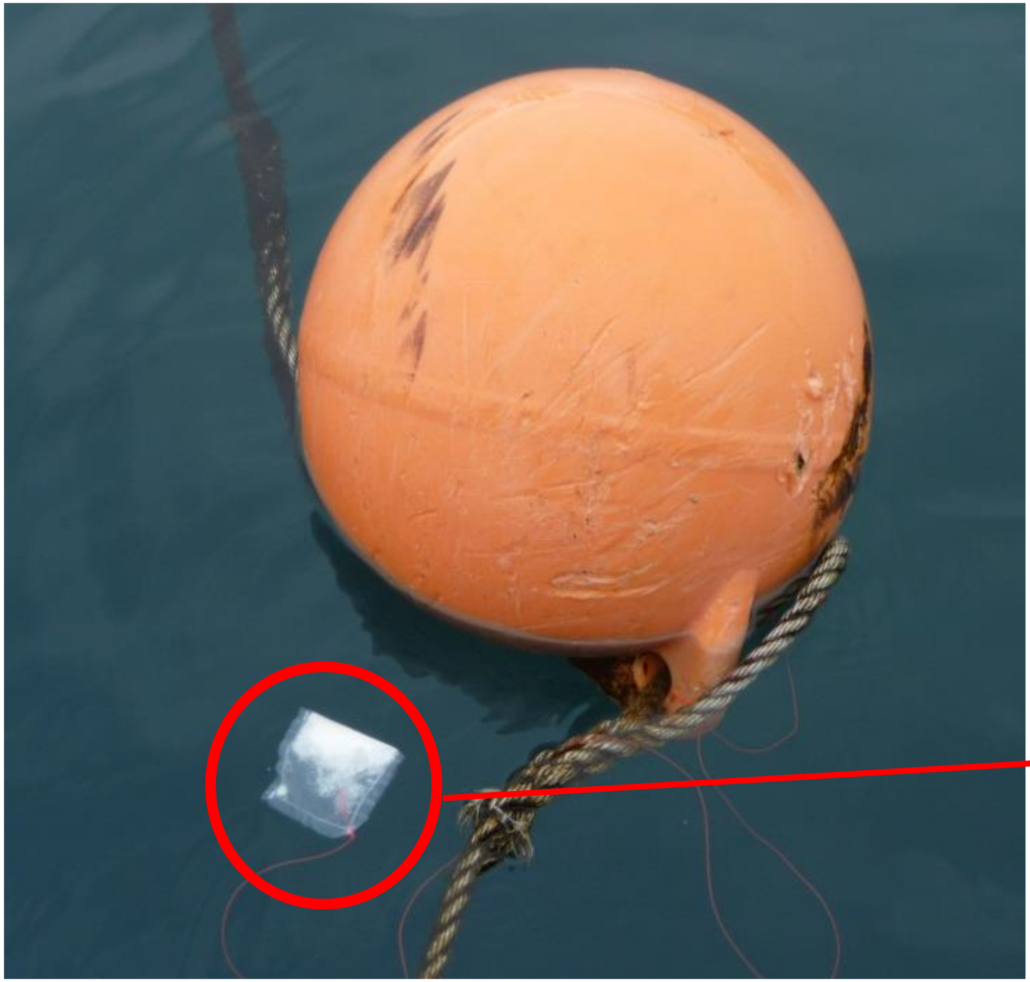

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HAB species range expansion?

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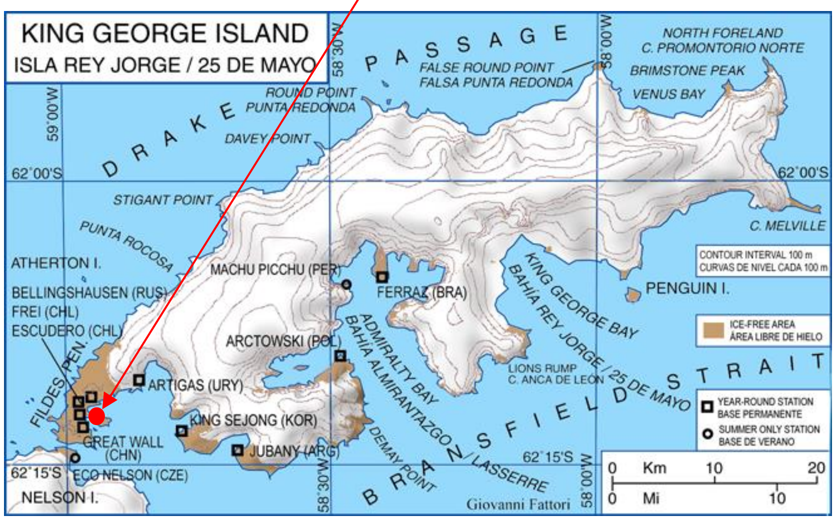

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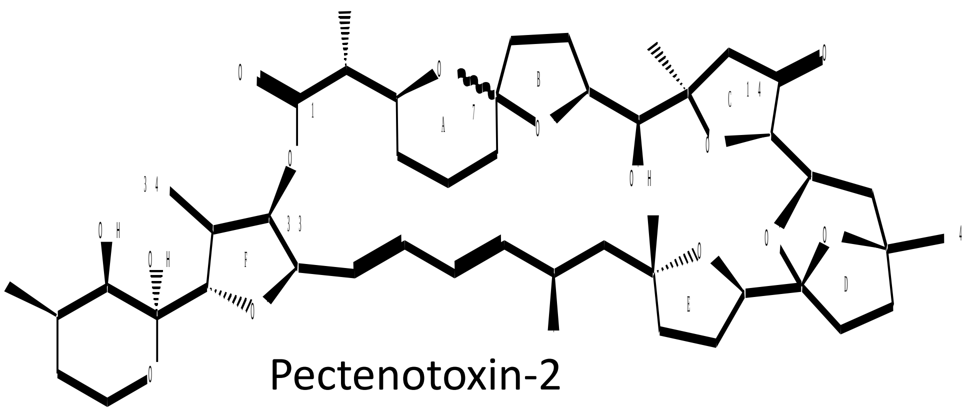
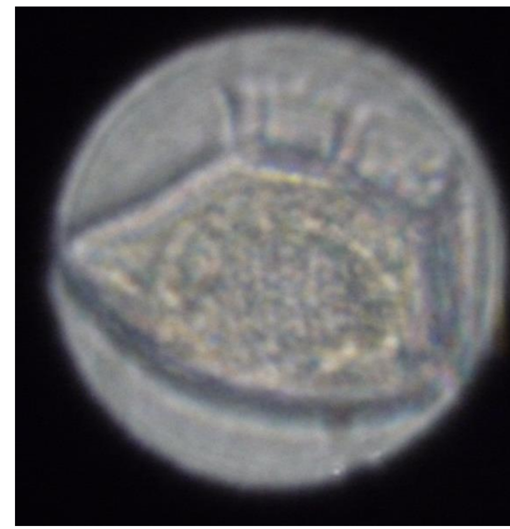
Plankton Net Haul



Pectenotoxin-2



Dinophysis norvegica

Dinophysis norvegica recently has been found in the SW Atlantic

Fabro et al. 2016. Harmful Algae 59,31-41.

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Ichthyotoxic HAB Events

- Gill damage
- O_2 defficiency
- secondary infections
- **Ichthyotoxins**

Ichthyotoxic HAB species

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Raphidophyceans/Dictyochophyceans:

Chattonella	
antiqua	Ichthyotoxins
globosa	Ichthyotoxins
marina	Ichthyotoxins
subsalsa	Ichthyotoxins
verruculosa	Ichthyotoxins
Fibrocapsa	
japonica	Ichthyotoxins
Heterosigma	
akashiwo	Ichthyotoxins
Pseudochattonella	
c.f. *veruculosa*	Ichthyotoxins

Chattonella antiqua

Fibrocapsa japonica

Heterosigma akashiwo

Toxins and exact mode of action unknown!

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Ichthyotoxic HAB species

Haptophytes:

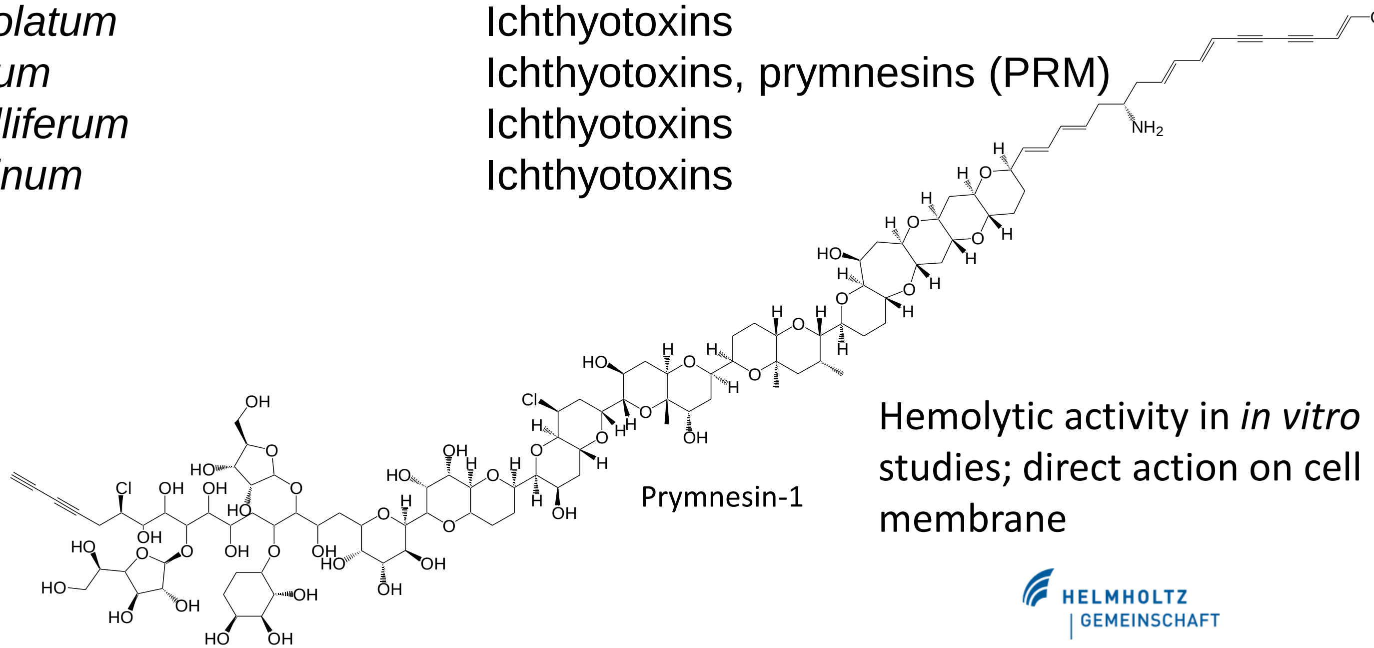
Chrysocromulina
- *leadbeateri* — Ichthyotoxins
- *polylepis* — Ichthyotoxins

Phaeocystis
- *pouchetii* — Ichthyotoxins (?)

Prymnesium
- *calathiferum* — Ichthyotoxins
- *faveolatum* — Ichthyotoxins
- *parvum* — Ichthyotoxins, prymnesins (PRM)
- *patelliferum* — Ichthyotoxins
- *zebrinum* — Ichthyotoxins


Chrysochromulina polylepis


Prymnesium parvum


Prymnesin-1

Hemolytic activity in *in vitro* studies; direct action on cell membrane

Toxins and exact mode of action unknown!

Ichthyotoxic HAB species

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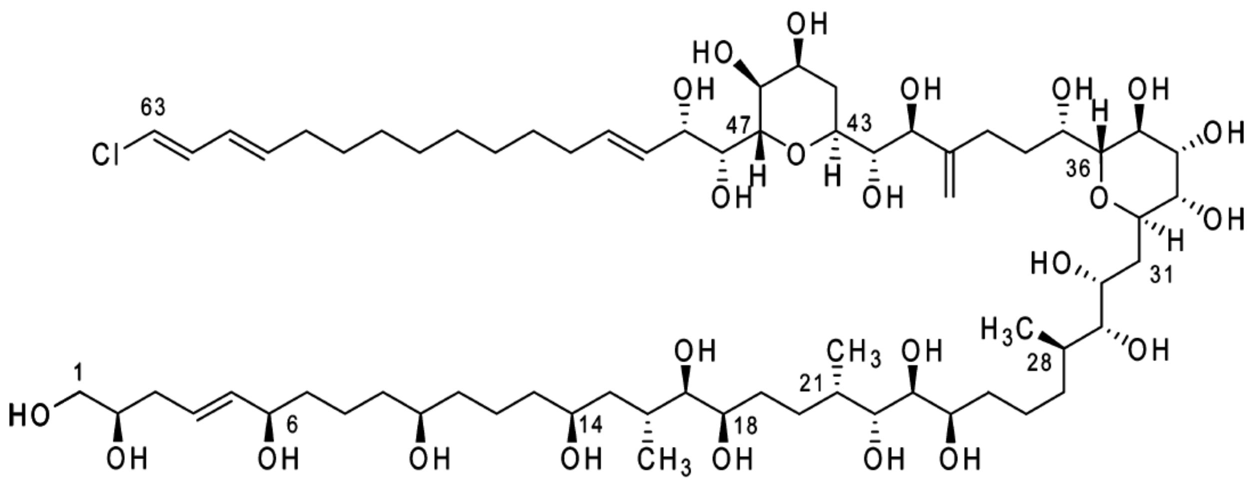
Naked (unarmoured) dinoflagellates:

Karlodinium
armiger
veneficum



Karlotoxin-2; M= 1344 g mol^{-1} (Peng et al. 2010)

Karlodinium veneficum

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Ichthyotoxic HAB species

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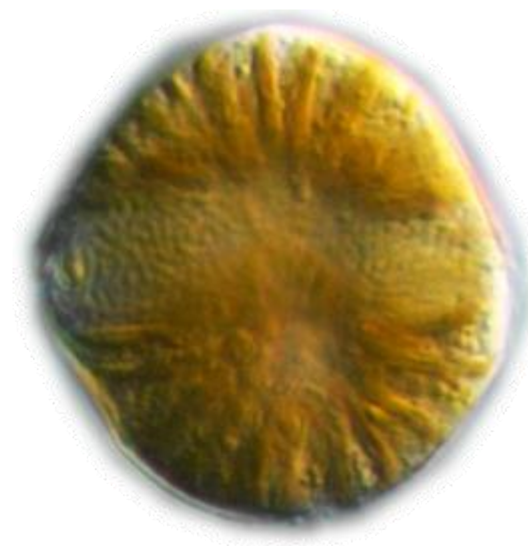
Thecate dinoflagellates:

Alexandrium
catenella
ostenfeldii
Protoceratium
reticulatum



Alexandrium ostenfeldii

Ichthyotoxins ?

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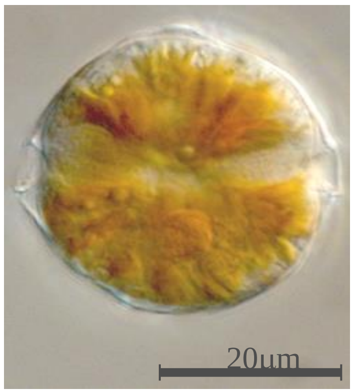
Ichthyotoxic HAB species

Lytic Effect



Alexandrium catenella strain 2 (Alex2)

Rhodomonas salina

Rhodomonas salina exposed to *A. catenella* supernatant (cell free)

Video: U. Tillmann

Ichthyotoxic HAB species

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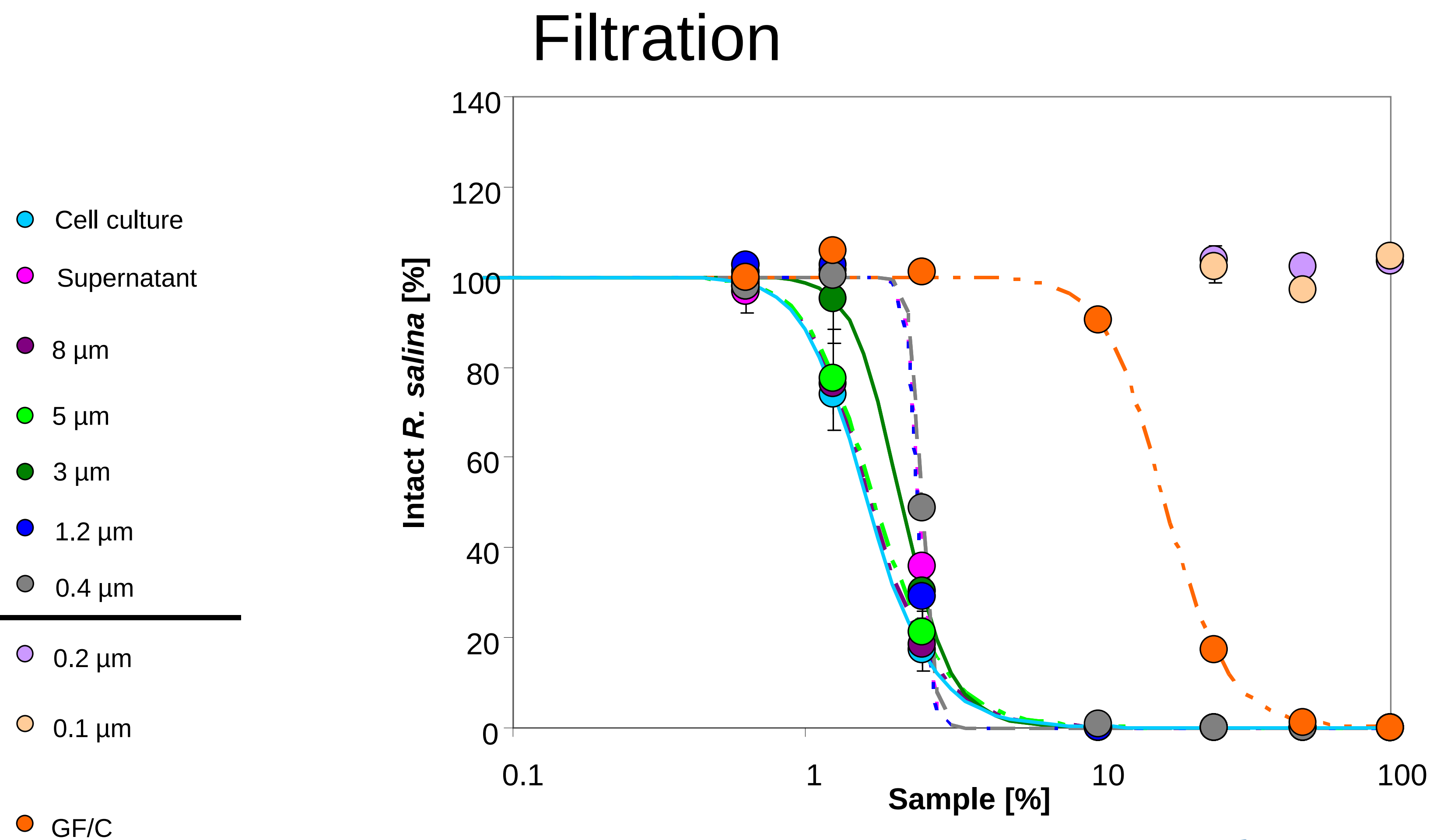
Characteristics



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Ichthyotoxic HAB species

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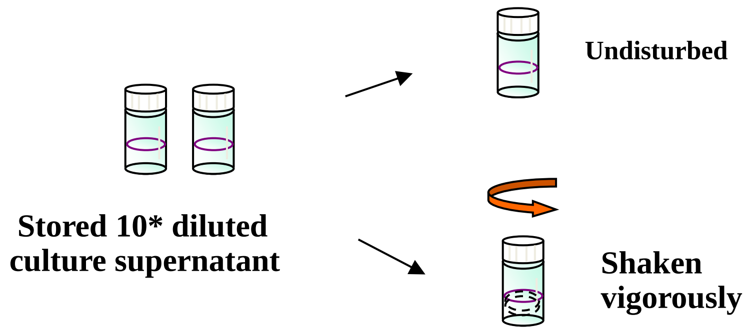
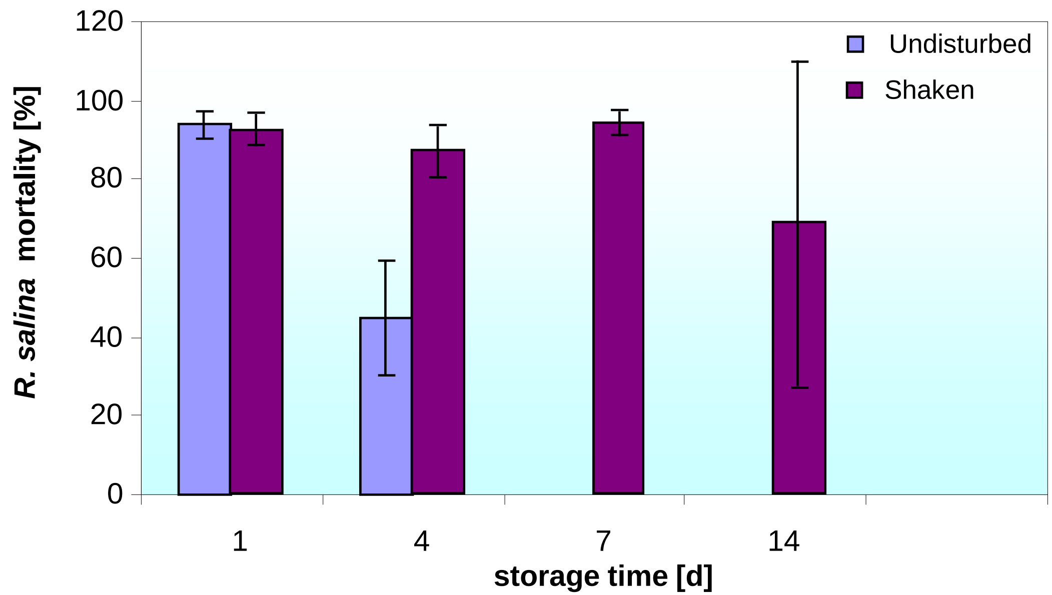
Characteristics

Time





Lytic activity ***cannot*** be extracted by organic solvents from the aqueous supernatant,

but is enriched in a foamy emulsion between the aqueous and organic phases.

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Ichthyotoxic HAB species

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Characteristics

- Liquid-liquid partitioning of supernatant (pH2) with n-hexane => no activity was extracted into the organic phase => **no lipids**
- Phenol sulfuric acid assay: saccharide content <2% => **no polysaccharides**
- Tryptic digestion did not change chromatographic behavior of lytic activity => **no proteins**

Ma et al. 2011. Harmful Algae 11, 65-72.

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Ichthyotoxic HAB species

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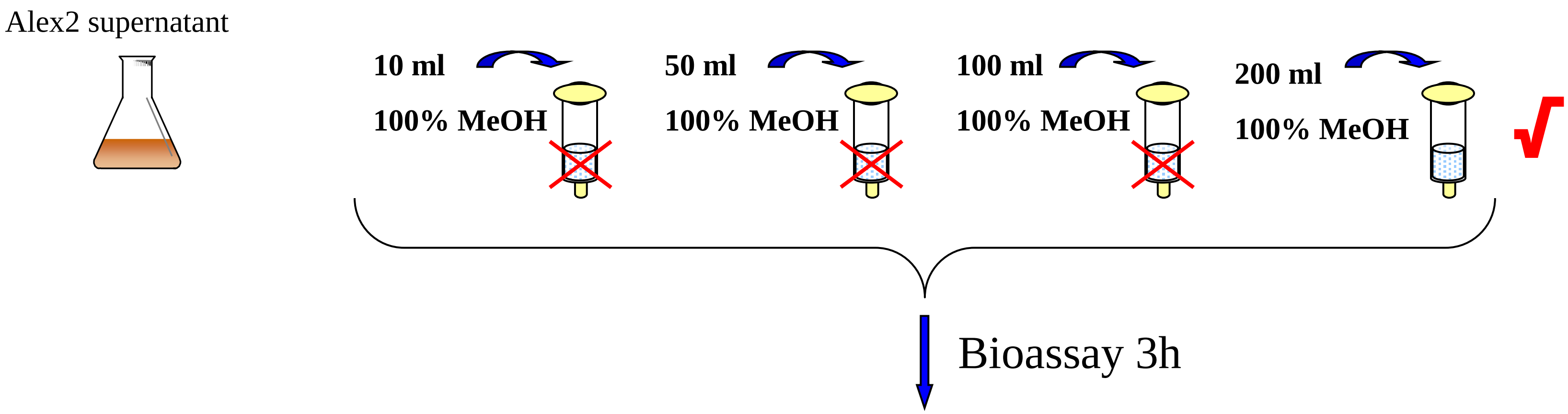
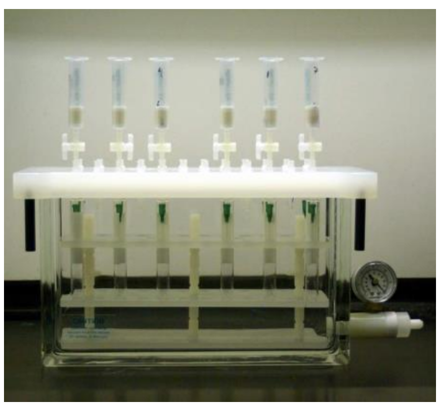
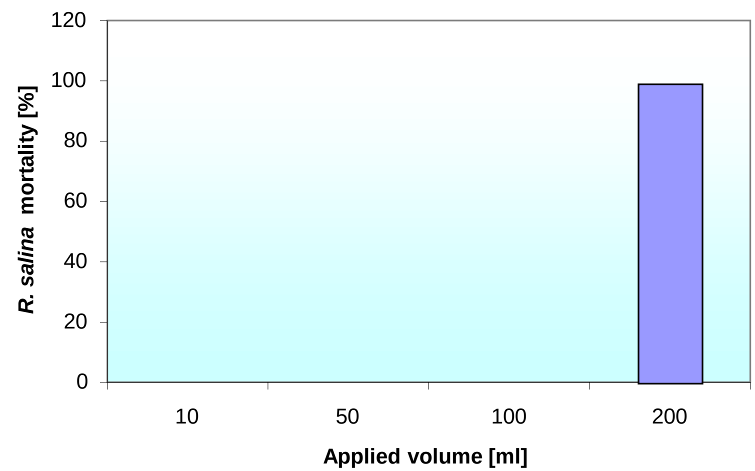
Purification: Reversed phase SPE





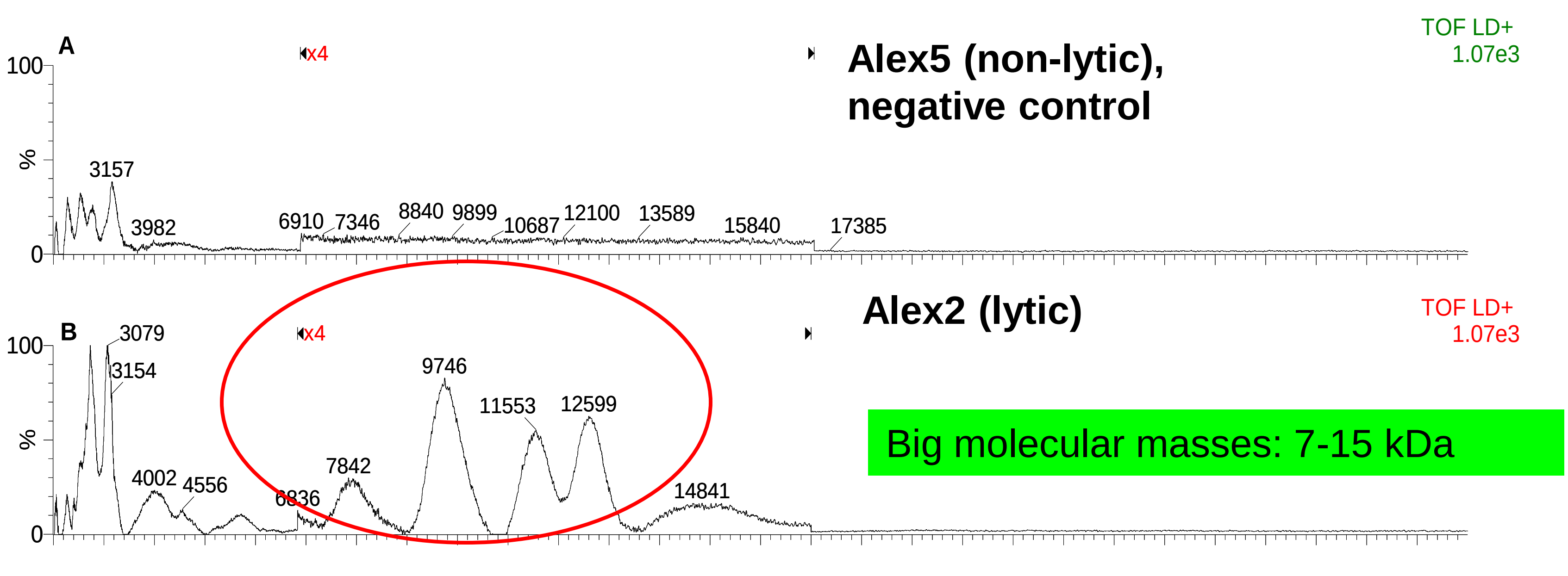


Allelochemicals „search“ their targets, i.e. specifically adsorb to lipophilic surfaces such as membranes

Ichthyotoxic HAB species

Mass spectrometric characterization

Time of Flight (TOF)-MS



MALDI-TOF mass spectra of HILIC fractions

Ichthyotoxic HAB species

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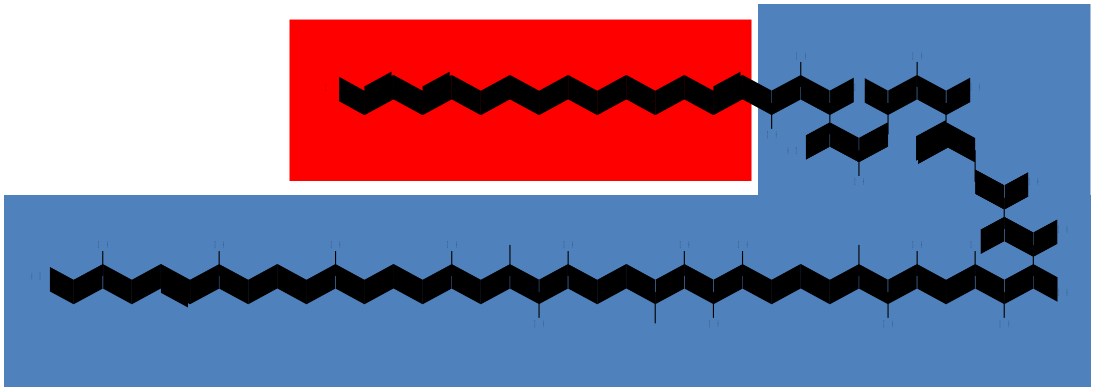
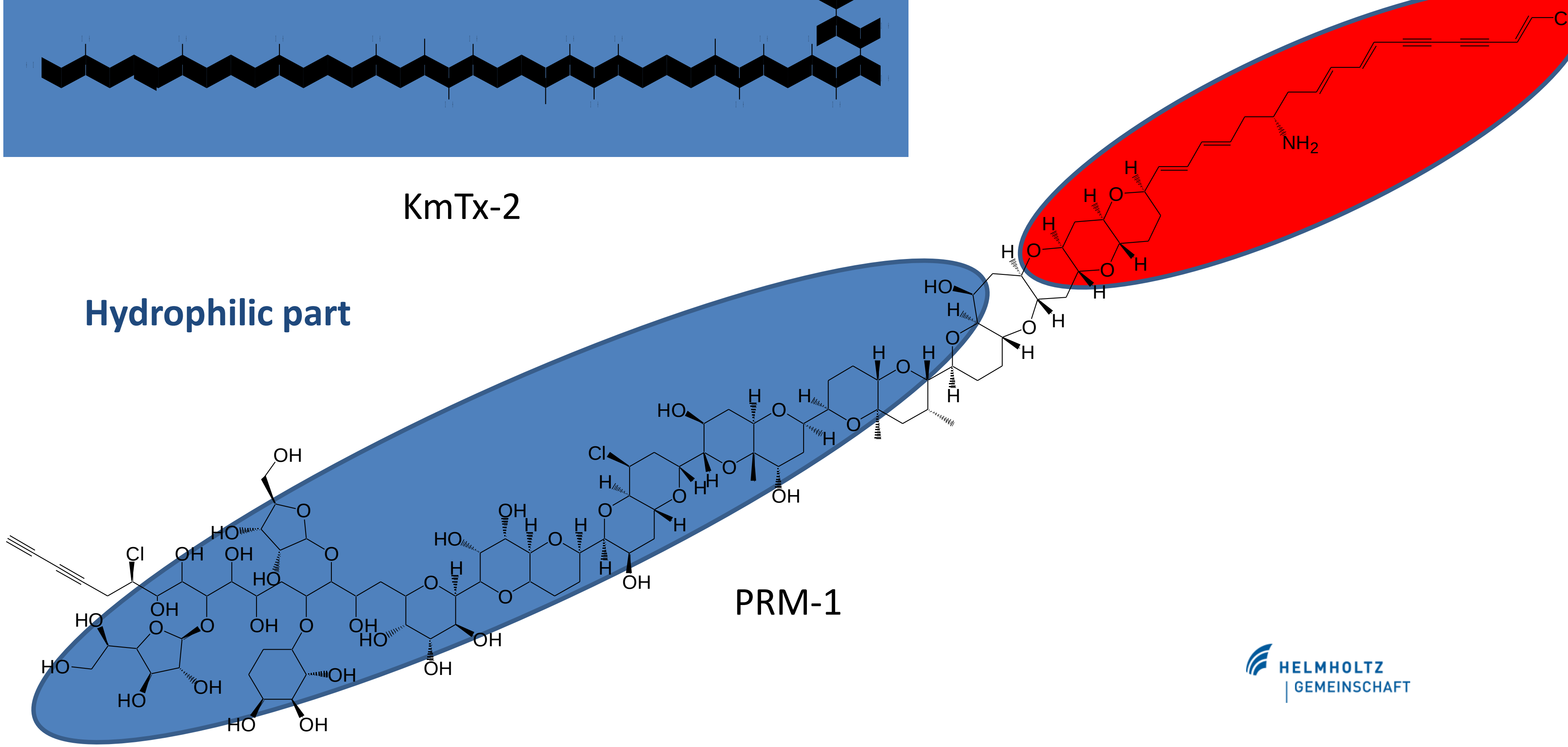


KmTx-2

Lipophilic part

Hydrophilic part



PRM-1

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Ichthyotoxic HAB species

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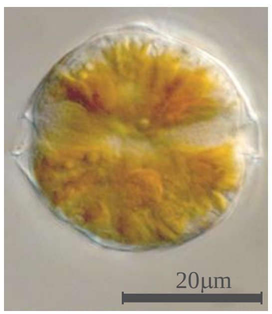

Alexandrium

- Paralytic shellfish poisoning *(Prakash et al., 1971)*
- Spirolides *(Cembella et al., 2000).*
- Gymnodimines *(Van Wagoner et al., 2011; Van de Waal et al. 2015)*
- Large scale fish kills *(Mortensen, 1985; Cembella et al., 2002)*
- Marine mammals mortality and morbidities *(Durbin et al., 2002; Doucette et al., 2006)*
- Allelopathy: toward other microalgae *(Blanco and Campos, 1988; Arzul et al., 1999; Fistarol et al., 2004; Tillmann et al., 2007)* and heterotrophic protists *(Hansen, 1989; Hansen et al., 1992; Matsuoka et al., 2000; Tillmann and John, 2002, Tillmann et al., 2007)*.

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Take home messages

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- Toxin variability can be very high *within* a local population but also *among* populations of different geographic regions
  => it is important to know local species and toxin variants

- Plankton communities may rapidly change over time in a given region
  => regular plankton monitoring including less abundant species is necessary

- Is ichthyotoxicity a side effect of interplanktonic defense mechanisms?

- Ichtyotoxins/allelochemicals need to be characterized to understand their fish killing activity and to develop mitigation strategies.

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¡Gracias por su atención!