



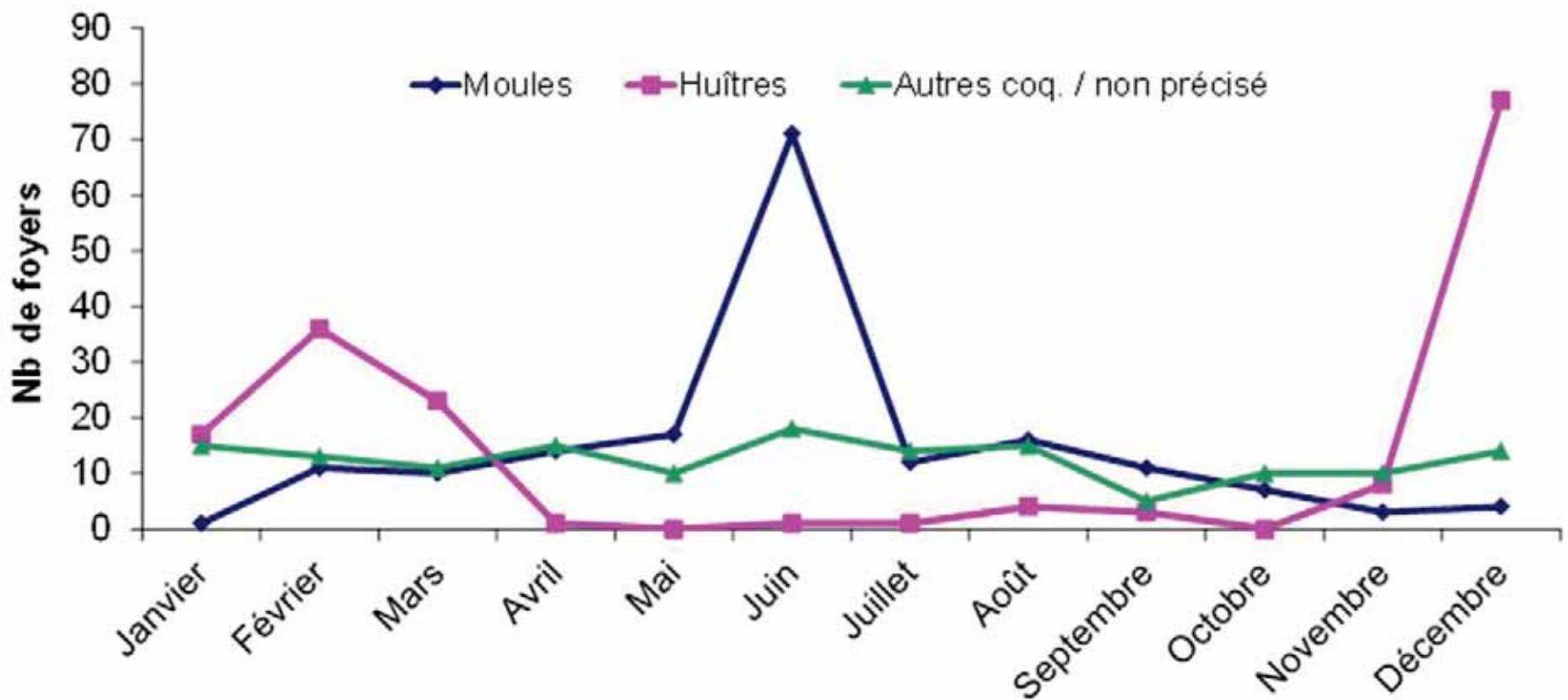
# TOXINES DE MICRO-ALGUES MARINES : UN DÉFI POUR LA SANTÉ HUMAINE ET L'ENVIRONNEMENT

PHILIPP HESS  
IFREMER, CENTRE ATLANTIQUE  
LABORATOIRE PHYCOTOXINES

# Contenu

- Introduction
- Cibles biologiques chez l'homme
- Diversité chimique des genres
- Changement Climatique
- Détection non-ciblée
- Perspectives

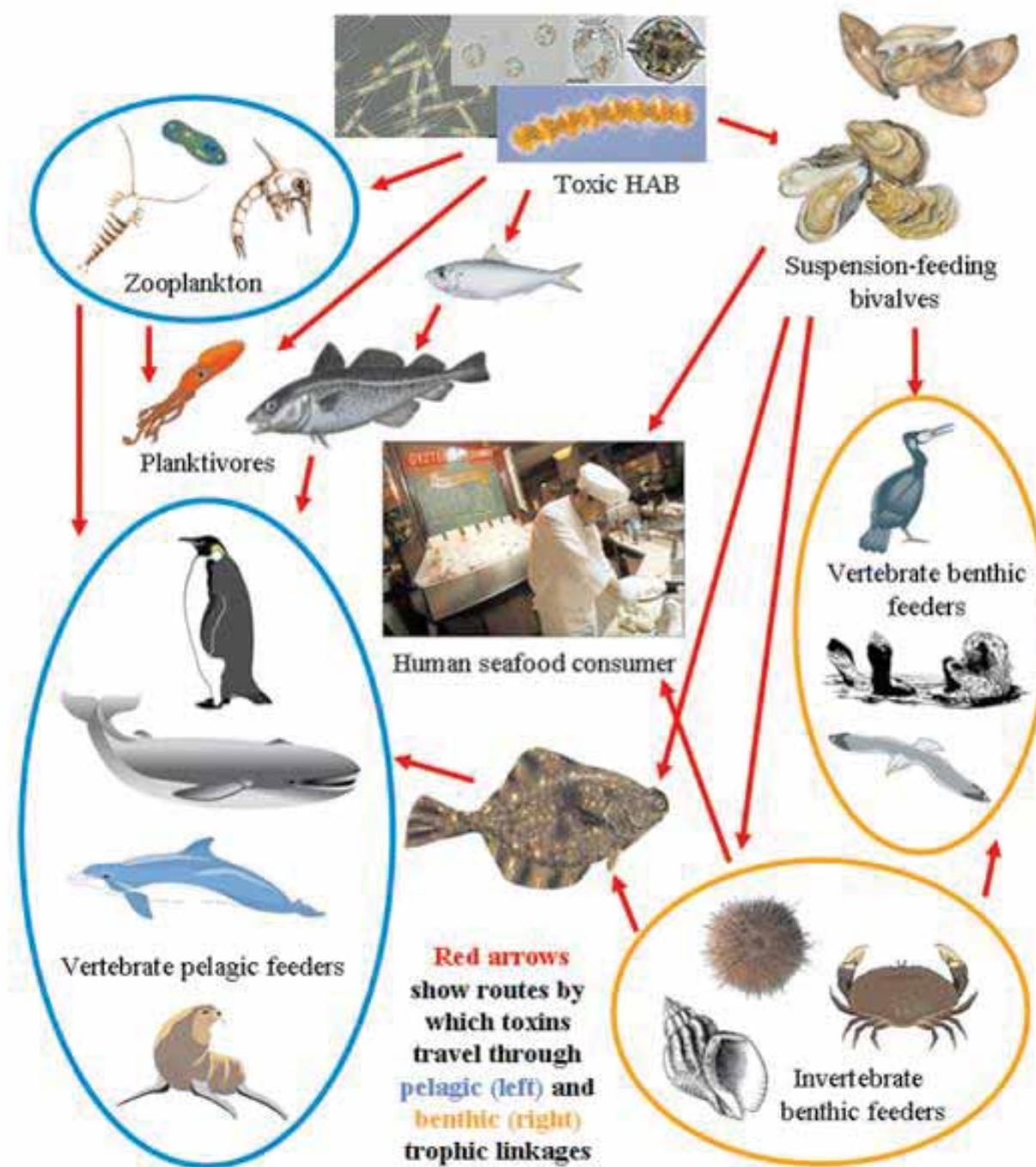
# Santé publique



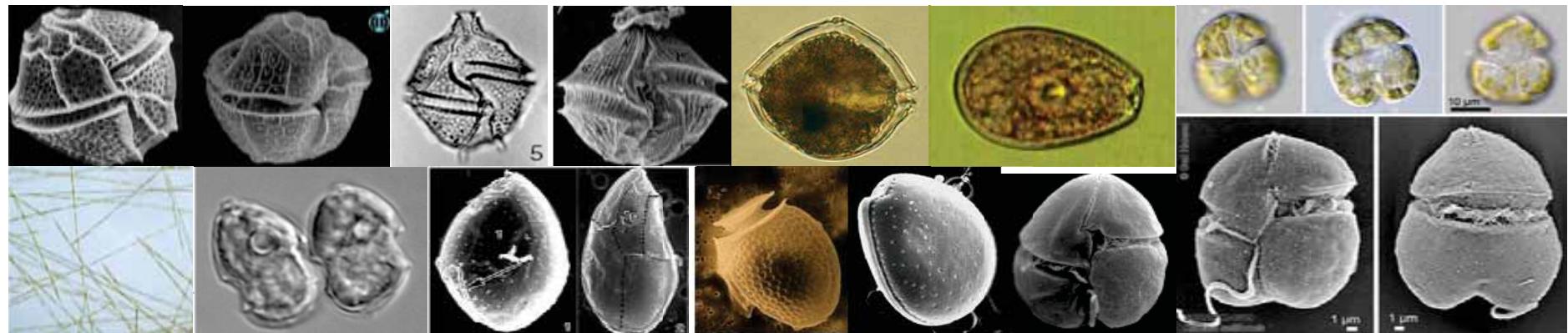
# Environnement



# Chaînes trophiques marines

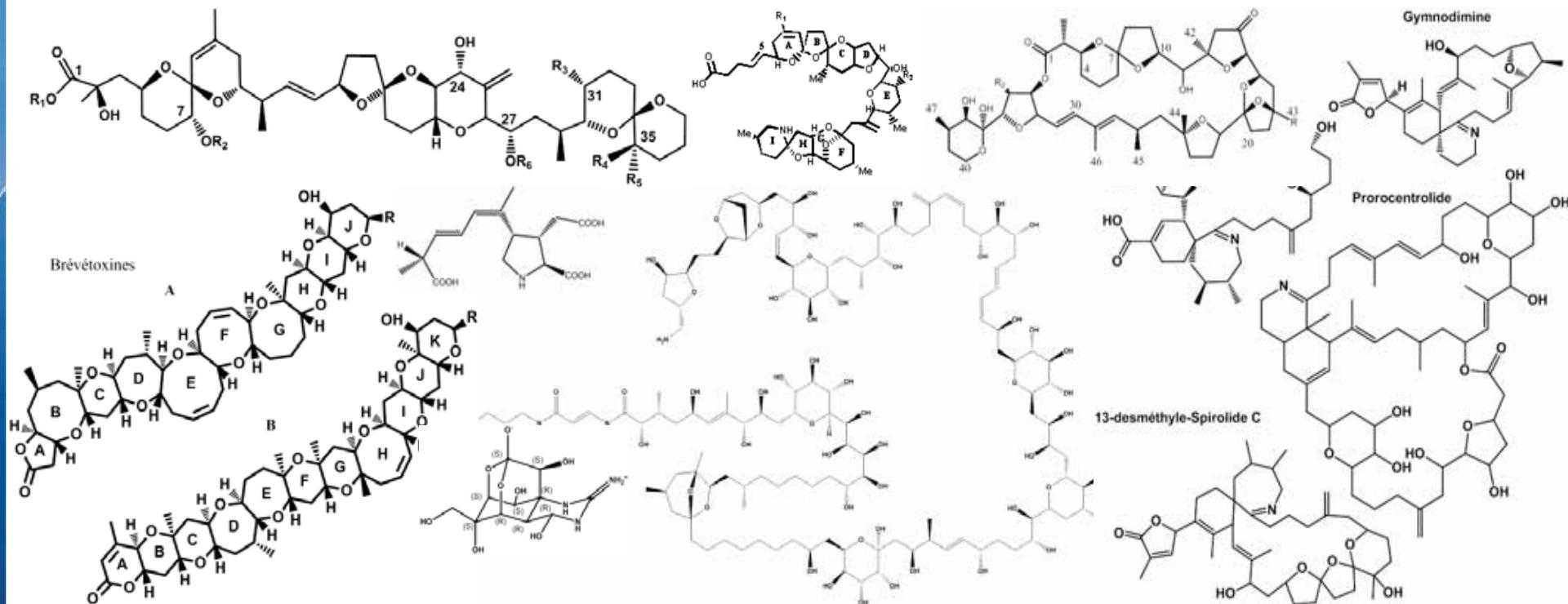


# Algues unicellulaires -Biodiversité



HAB  
Red tides  
Green tides  
Brown tides

# Toxines - Chimiodiversité



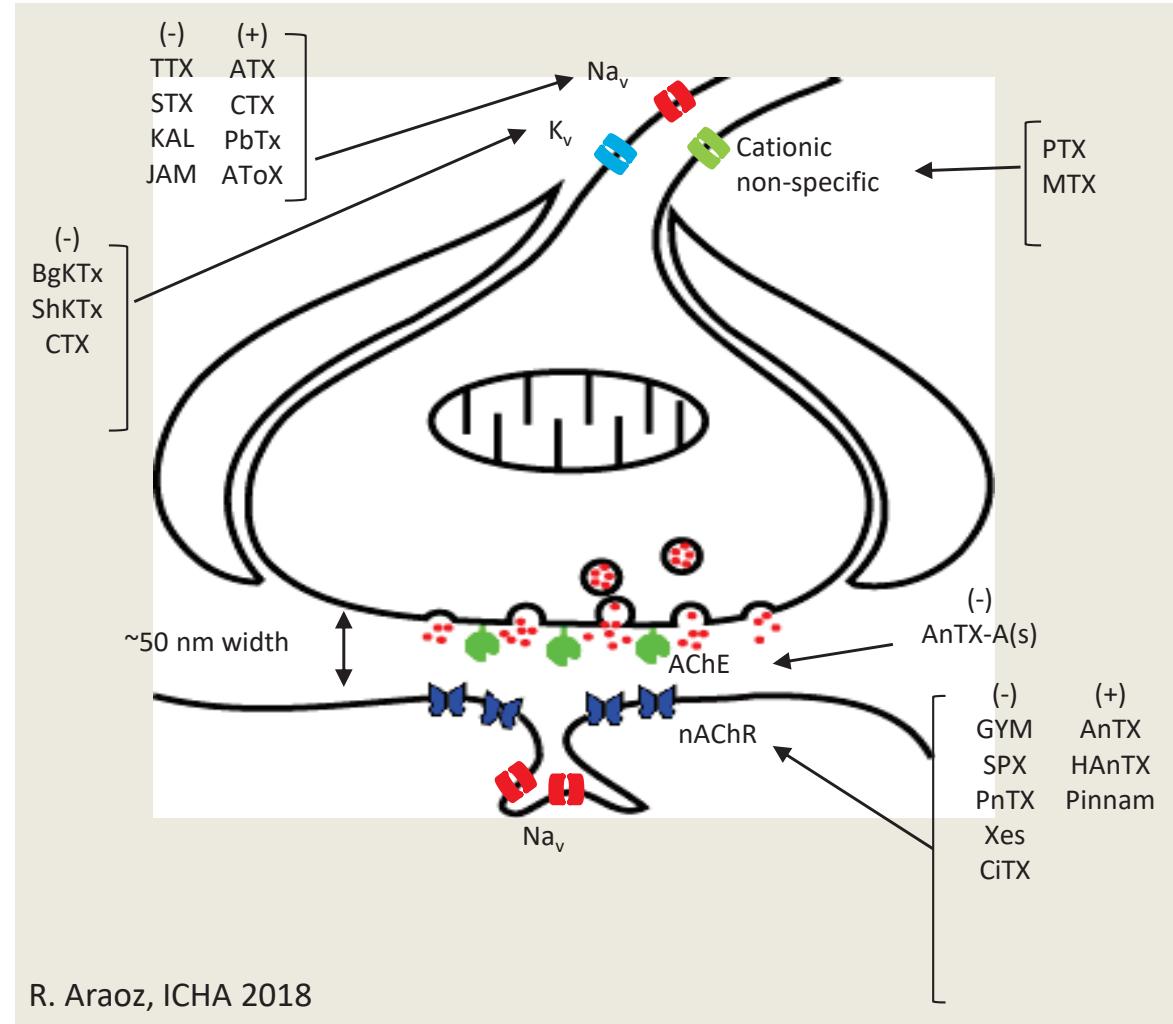
## Produits naturels

Alcaloïdes  
Acides aminés  
Imines cycliques  
Polycétides

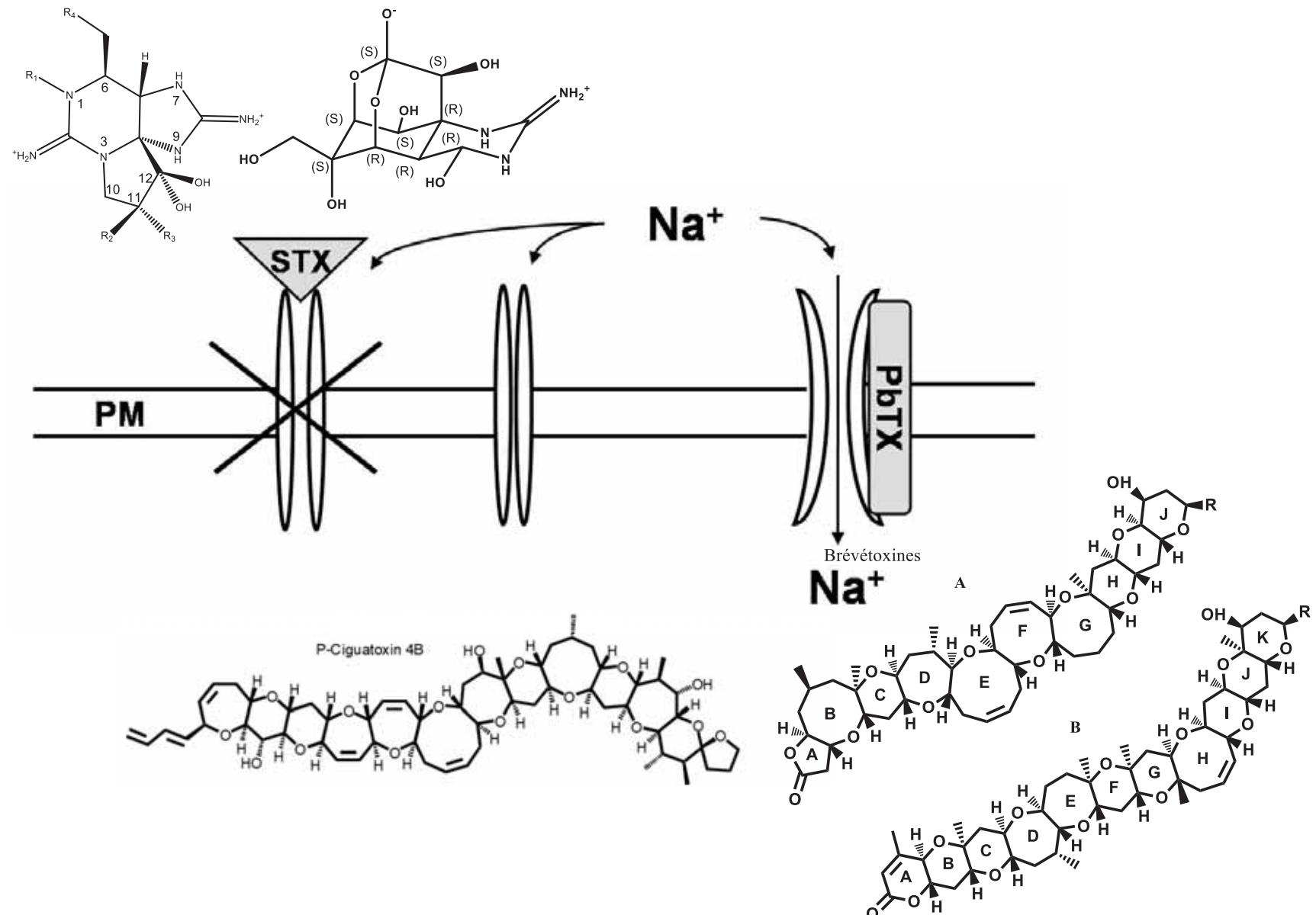
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# Transmission neuronale - jonction neuromusculaire



# Canaux sodiques



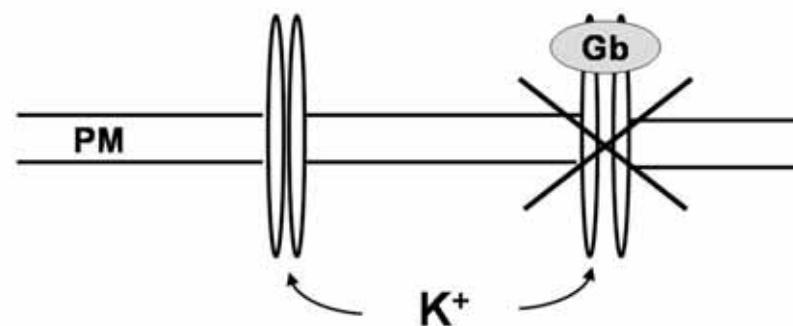
# Canaux sodiques



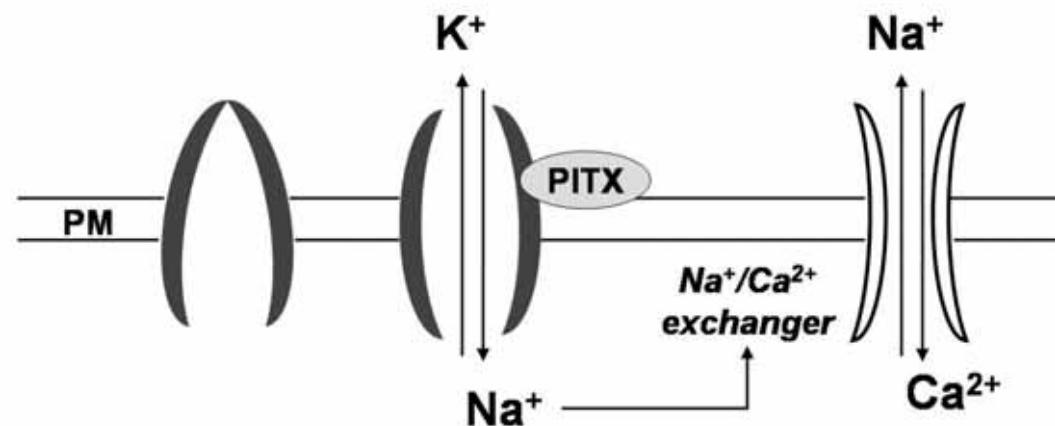
STX utilisées dans le traitement de la peine  
(e.g. peine chronique de la vessie chez la femme)

# Canaux ioniques

VGKC

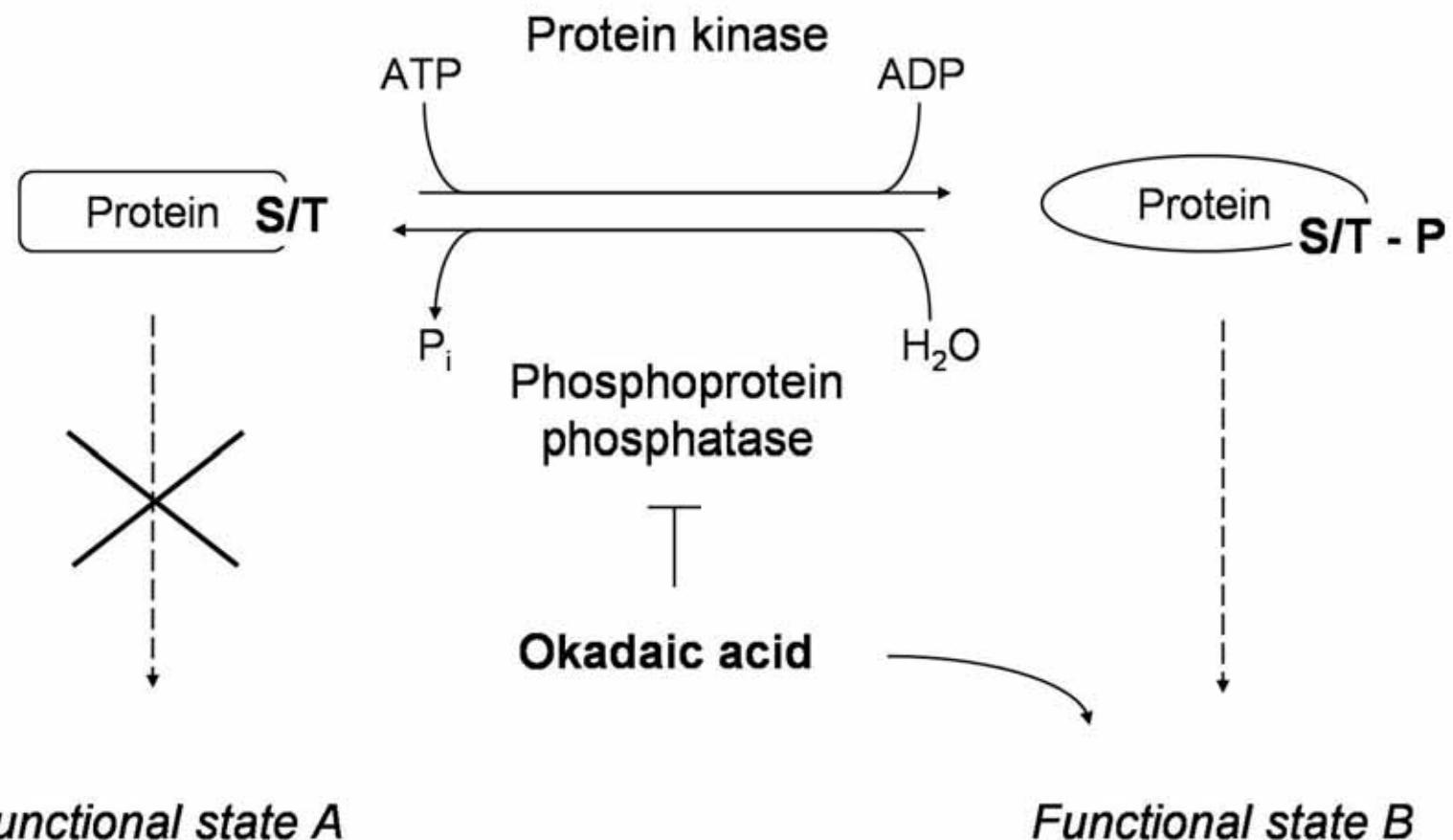


C

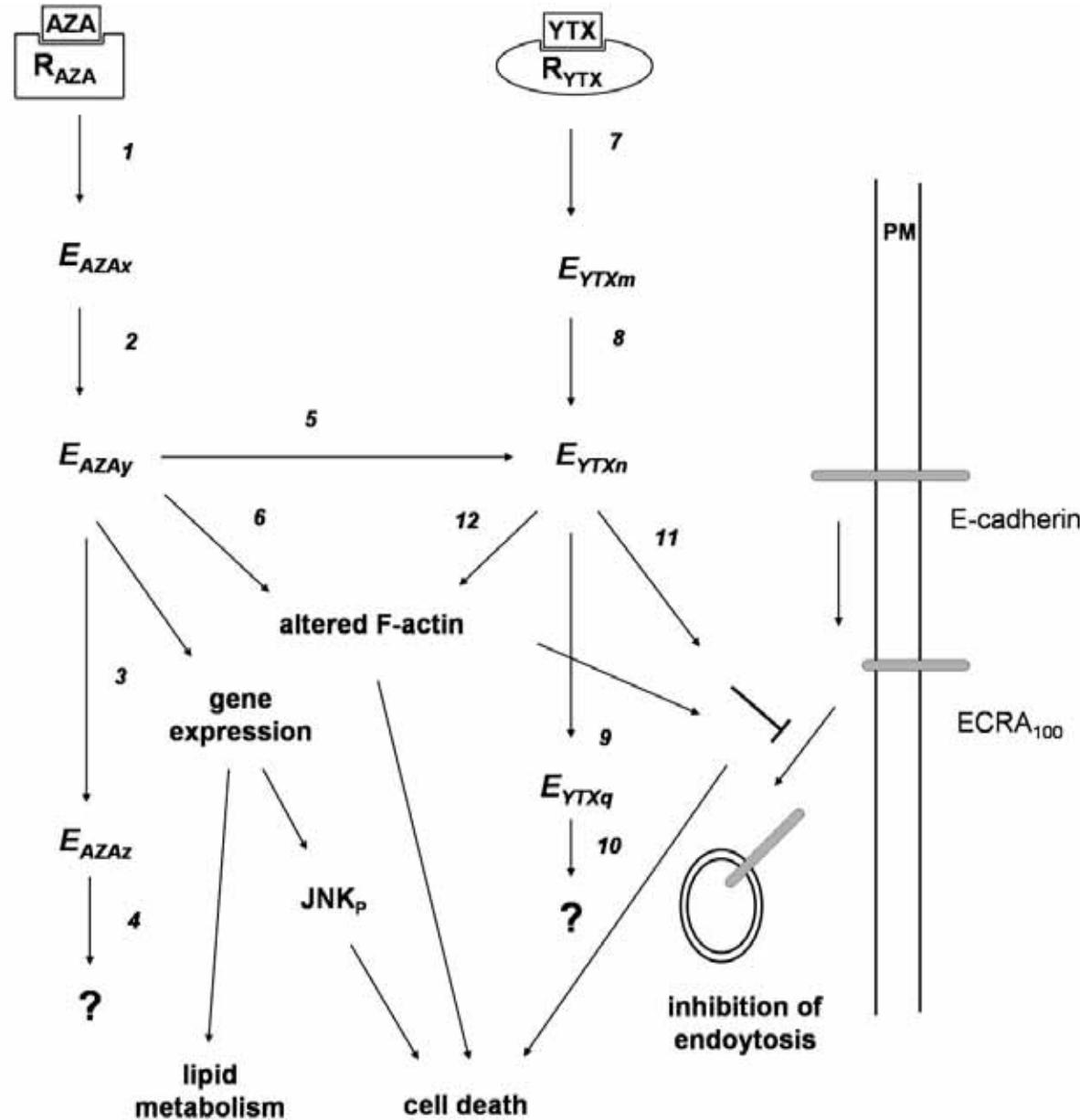
Na<sup>+</sup>, K<sup>+</sup> - ATPase

D

# Inhibition des Phosphoprotéines-phosphatases



# Mécanismes inconnus

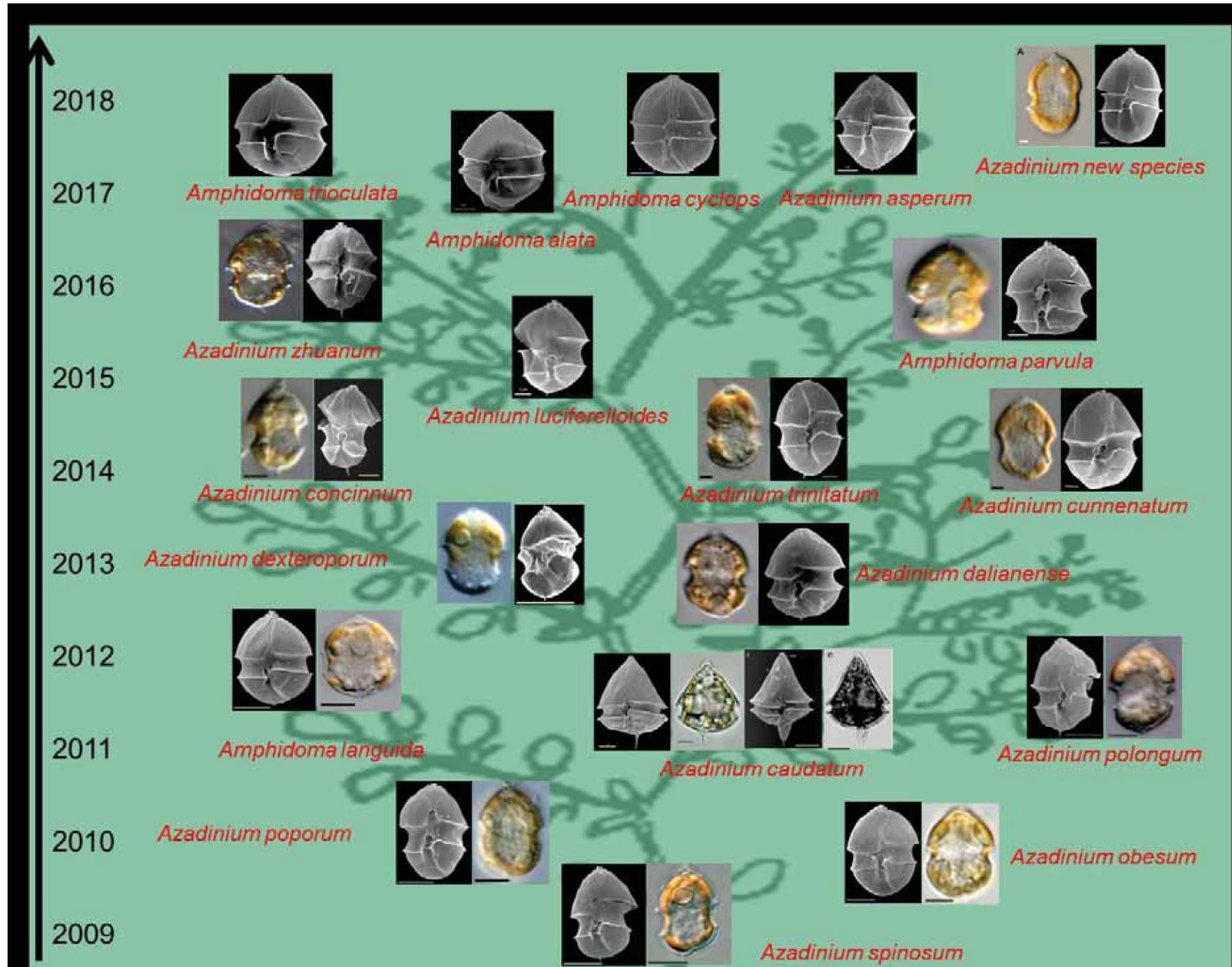


Pelin, M.; Kilcoyne, J.; Florio, C.; Hess, P.; Tubaro, A.; Sosa, S., **Azaspiracids Increase Mitochondrial Dehydrogenases Activity in Hepatocytes: Involvement of Potassium and Chloride Ions.** Mar. Drugs 2019, 17 (5), 276.

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# Biodiversité des genres *Azadinium* & *Amphidoma*

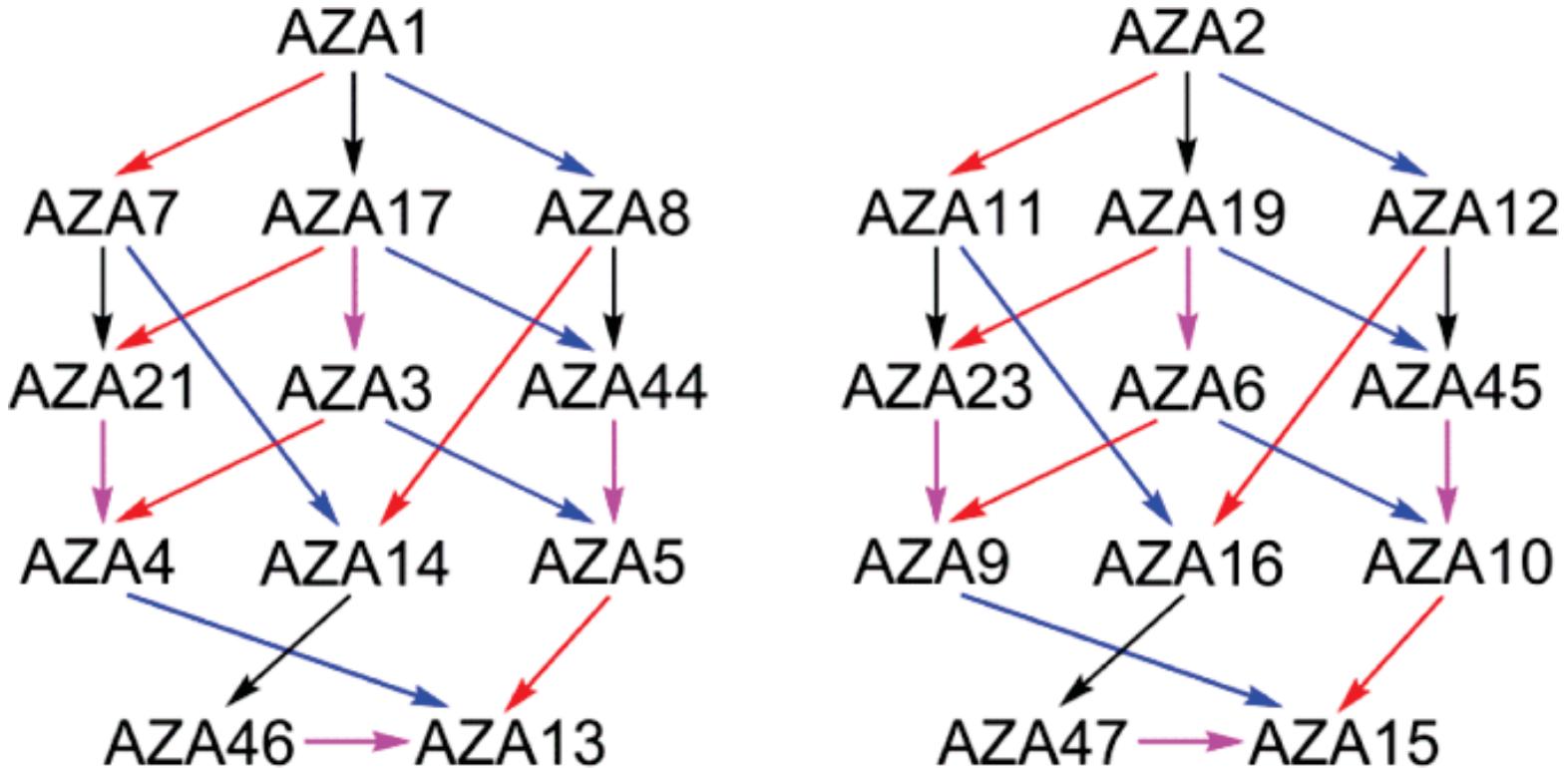


# Diversité chimique du genre *Azadinium*

#	AZA	<i>m/z</i> [M+H] <sup>+</sup>	<i>m/z</i> group 4 fragment	<i>m/z</i> group 5 fragment	Producer	Reference
1	AZA-1	842	362	262	<i>Az. spinosum</i>	Krock et al., 2009
2	AZA-2	856	362	262	<i>Az. spinosum</i>	Krock et al., 2009
					<i>Az. poporum</i>	Krock et al., 2014
					<i>Am. languida</i>	Tillmann et al., 2017a
3	epi-AZA-7	858	362	262	<i>Az. dexteroporum</i>	Rossi et al., 2017
4	AZA-11	872	362	262	<i>Az. spinosum</i>	Tillmann et al., 2018
					<i>Az. poporum</i>	Krock et al., 2014
5	AZA-33	716	362	262	<i>Az. spinosum</i>	Kilcoyne et al., 2014
6	AZA-34	816	362	262	<i>Az. spinosum</i>	Kilcoyne et al., 2014
7	AZA-35	830	362	262	<i>Az. spinosum</i>	Kilcoyne et al., 2014
					<i>Az. dexteroporum</i>	Rossi et al., 2017
8	AZA-36	858	348	248	<i>Az. poporum</i>	Krock et al., 2015
9	AZA-37	846	348	248	<i>Az. poporum</i>	Krock et al., 2015
10	AZA-38	830	348	248	<i>Am. languida</i>	Krock et al., 2012
11	AZA-39	816	348	248	<i>Am. languida</i>	Krock et al., 2012
12	AZA-40	842	348	248	<i>Az. poporum</i>	Krock et al., 2014
13	AZA-41	854	360	260	<i>Az. poporum</i>	Krock et al., 2014
14	AZA-42	870	360	260	<i>Az. poporum</i>	This work
15	AZA-43	828	360	260	<i>Am. languida</i>	Tillmann et al., 2017a
16	AZA-50	842	348	262	<i>Az. spinosum</i>	Tillmann et al., 2018
17	AZA-51	858	348	262	<i>Az. spinosum</i>	Tillmann et al., 2018
18	AZA-52	830	348	248	<i>Am. languida</i>	Tillmann et al., 2018
19	AZA-53	830	348	248	<i>Am. languida</i>	Tillmann et al., 2018
20	AZA-54	870	362	262	<i>Az. dexteroporum</i>	Rossi et al., 2017
21	AZA-55	868	360	260	<i>Az. dexteroporum</i>	Rossi et al., 2017
22	AZA-56	884	362	262	<i>Az. dexteroporum</i>	Rossi et al., 2017
23	AZA-57	844	362	262	<i>Az. dexteroporum</i>	Rossi et al., 2017
24	AZA-58	828	362	262	<i>Az. dexteroporum</i>	Rossi et al., 2017
25	AZA-59	860	362	262	<i>Az. poporum</i>	Kim et al., 2017
26	AZA-62	870	362	262	<i>Az. poporum</i>	This work

Krock et al., 2019 Two novel azaspiracids from *Azadinium poporum*, and a comprehensive compilation of azaspiracids produced by Amphidomataceae, (Dinophyceae). Harmful Algae 82, 1-8.

# Biotransformation

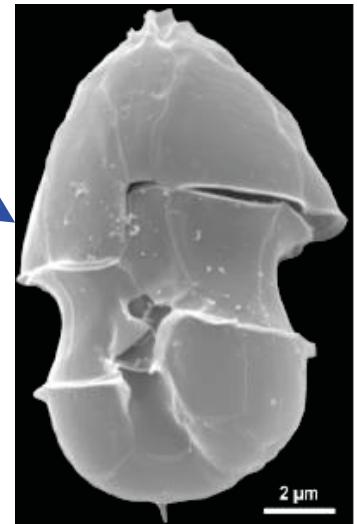
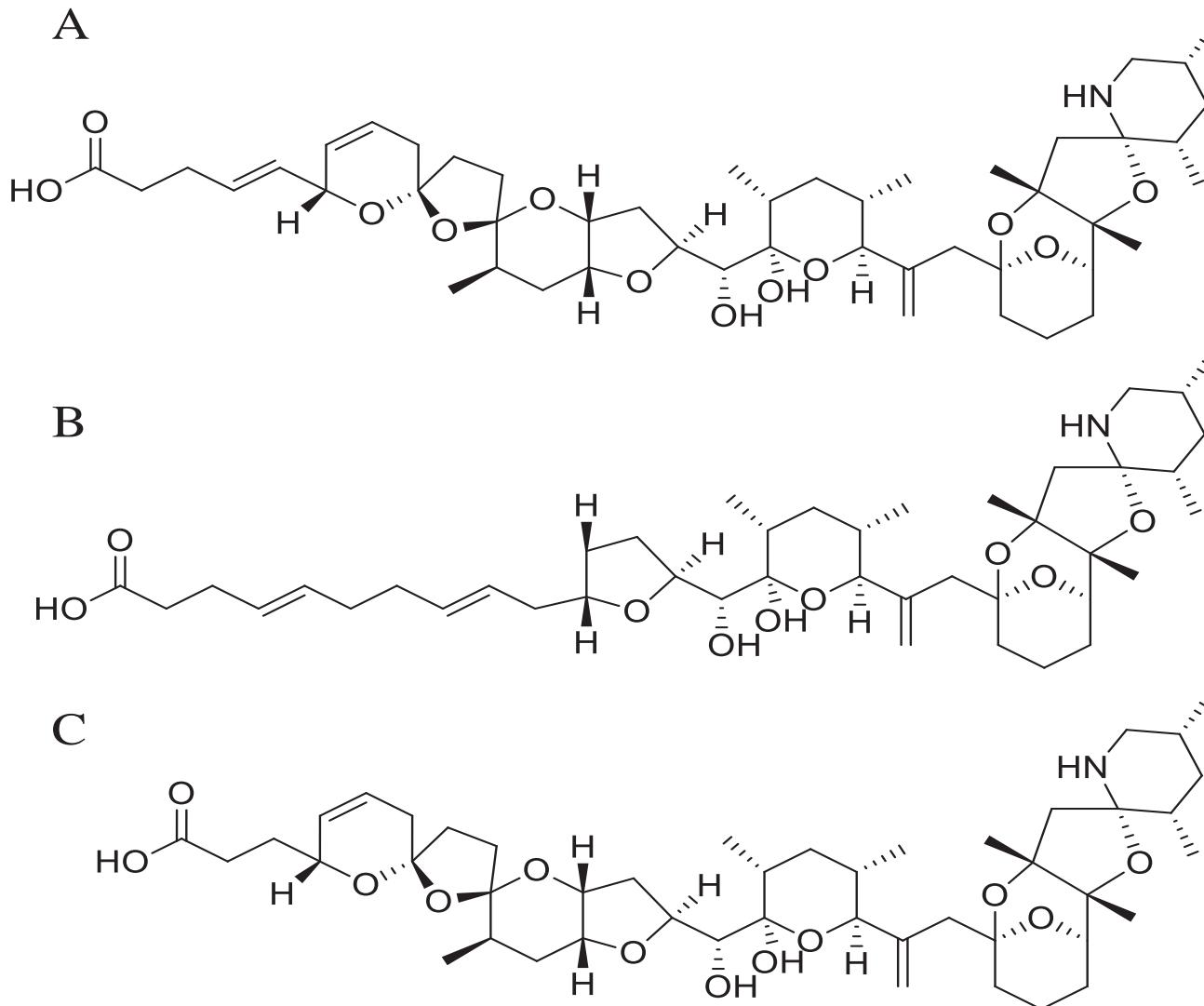


- Hydroxylation at C-3
- Hydroxylation at C-23
- Oxidation of 22-Me
- 22-Decarboxylation

Kilcoyne, J.; McCarron, P.; Hess, P.; Miles, C. O., Effects of Heating on Proportions of Azaspiracids 1–10 in Mussels (*Mytilus edulis*) and Identification of Carboxylated Precursors for Azaspiracids 5, 10, 13, and 15. *J. Agric. Food Chem.* 2015, 63 (51), 10980–10987.

# Différents composés biosynthétisés par :

A) AZA1, B) AZA33 and C) AZA34



***Azadinium  
spinosum***

AZA33 :

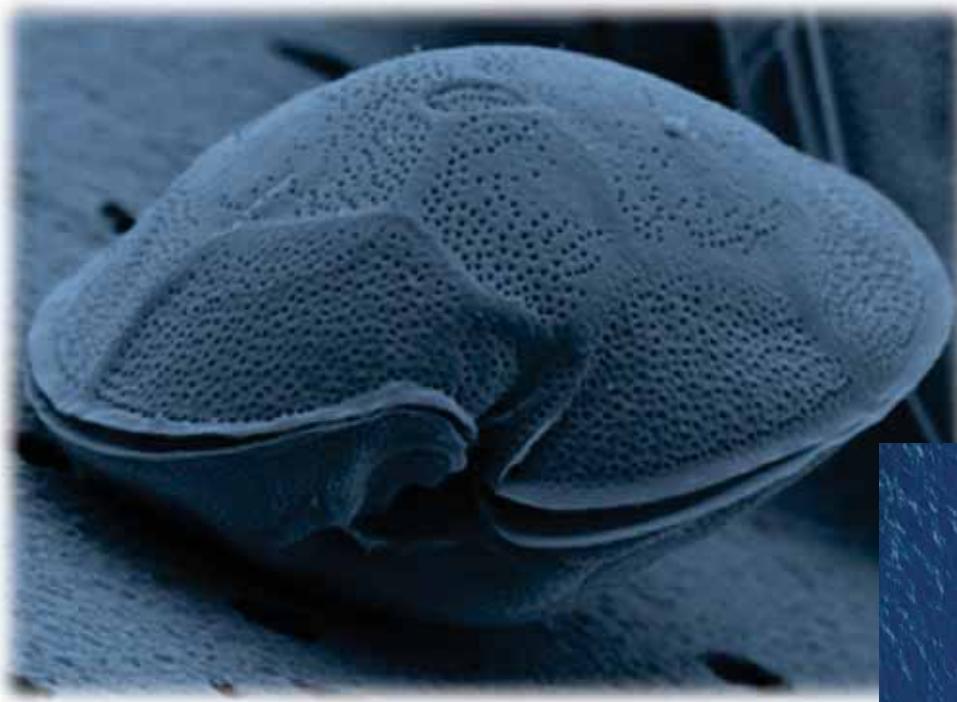
5-fold less  
toxic

AZA34:

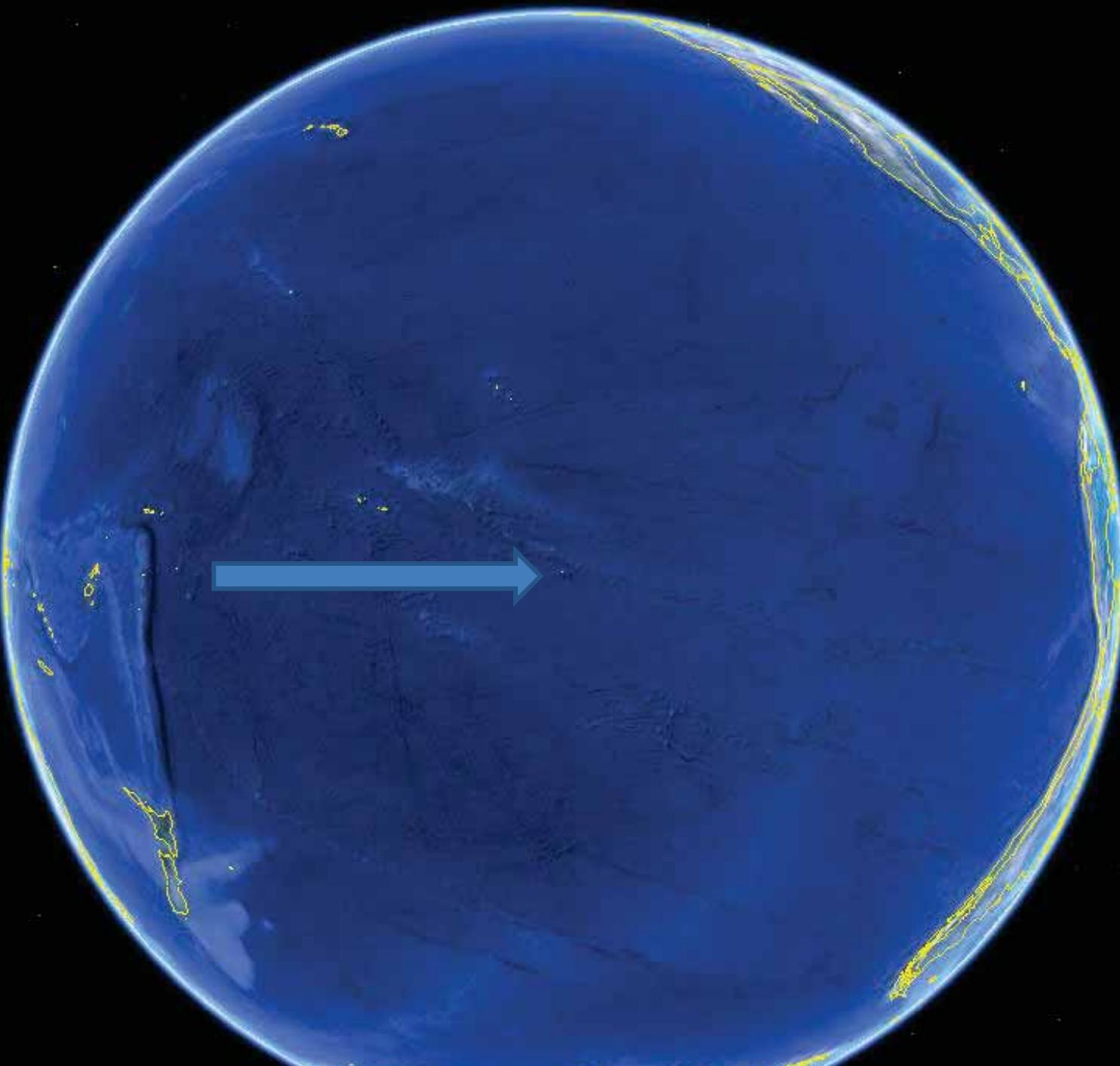
5-fold more  
toxic

Hess, P., et al., 2014. Azaspiracids, *Seafood and Freshwater Toxins*. CRC Press, pp. 799-822.  
Kilcoyne et al., 2014. *J. Nat. Prod.* 77 (11) 2465-2474.

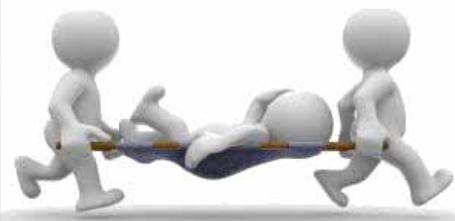
# Ciguatoxines - *Gambierdiscus*



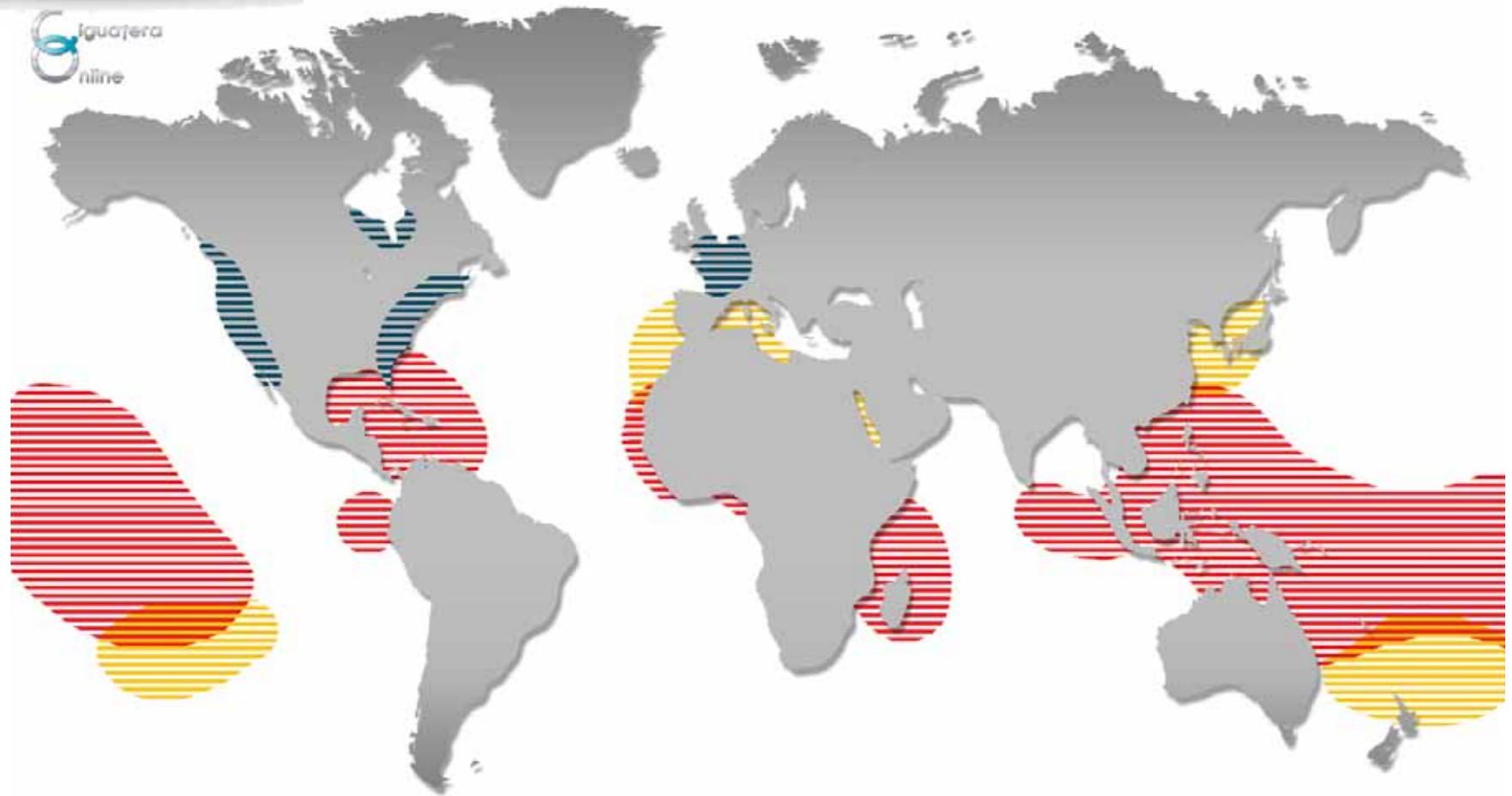
## Neurotoxine - ciguatéra



US Dept of State Geographer  
Data SIO, NOAA, U.S. Navy, NGA, GEBCO  
© 2012 Inav/Geosistemas SRL



# Ciguatera **Plus de 500 000 cas/an**

Ciguatera  
Online

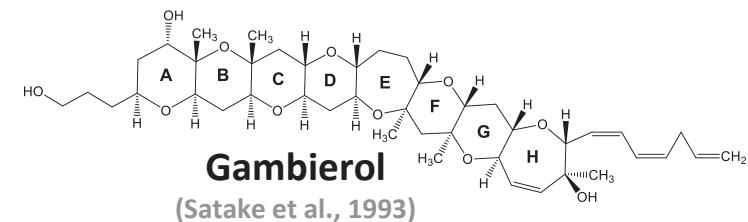
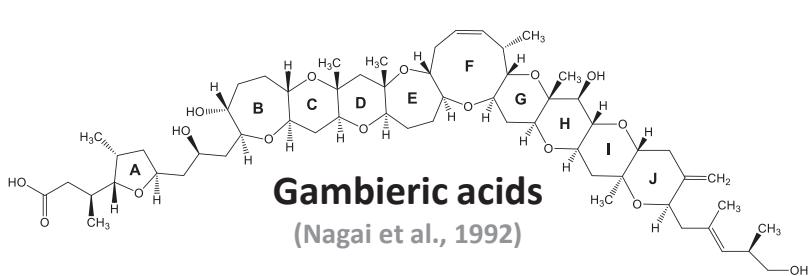
Zone d'endémie de la ciguatera

Cas «importés» de ciguatera

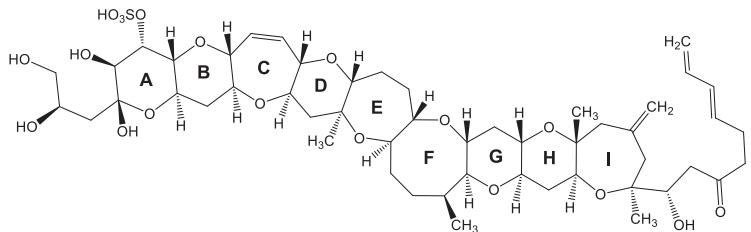
Nouvelles zones d'expansion de la ciguatera (depuis 2005)

- 2004 5 cas aux îles Canaries
- 2008 11 pêcheurs intoxiqués aux îles Sauvages (Azores)
- 2008 *Gambierdiscus spp* découvert dans la Méditerranée
- 2011 *Gambierdiscus excentricus* découvert aux îles Canaries

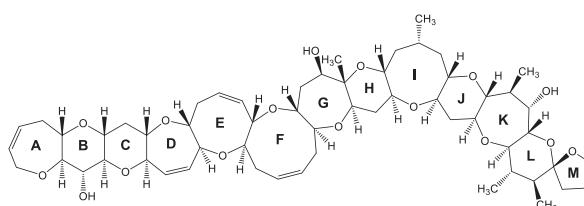
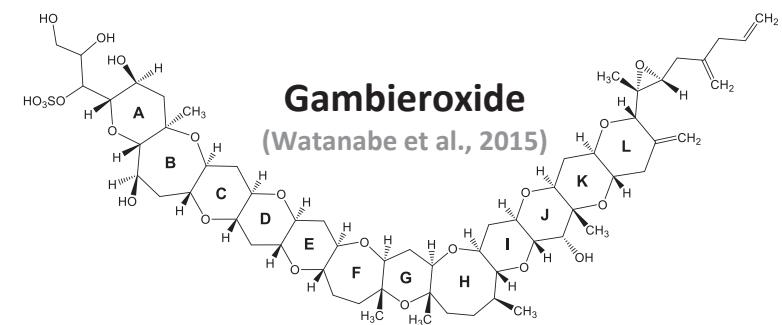
© 2014 Institut Louis Malardé



**Gambierone**  
(Rodríguez et al., 2015)

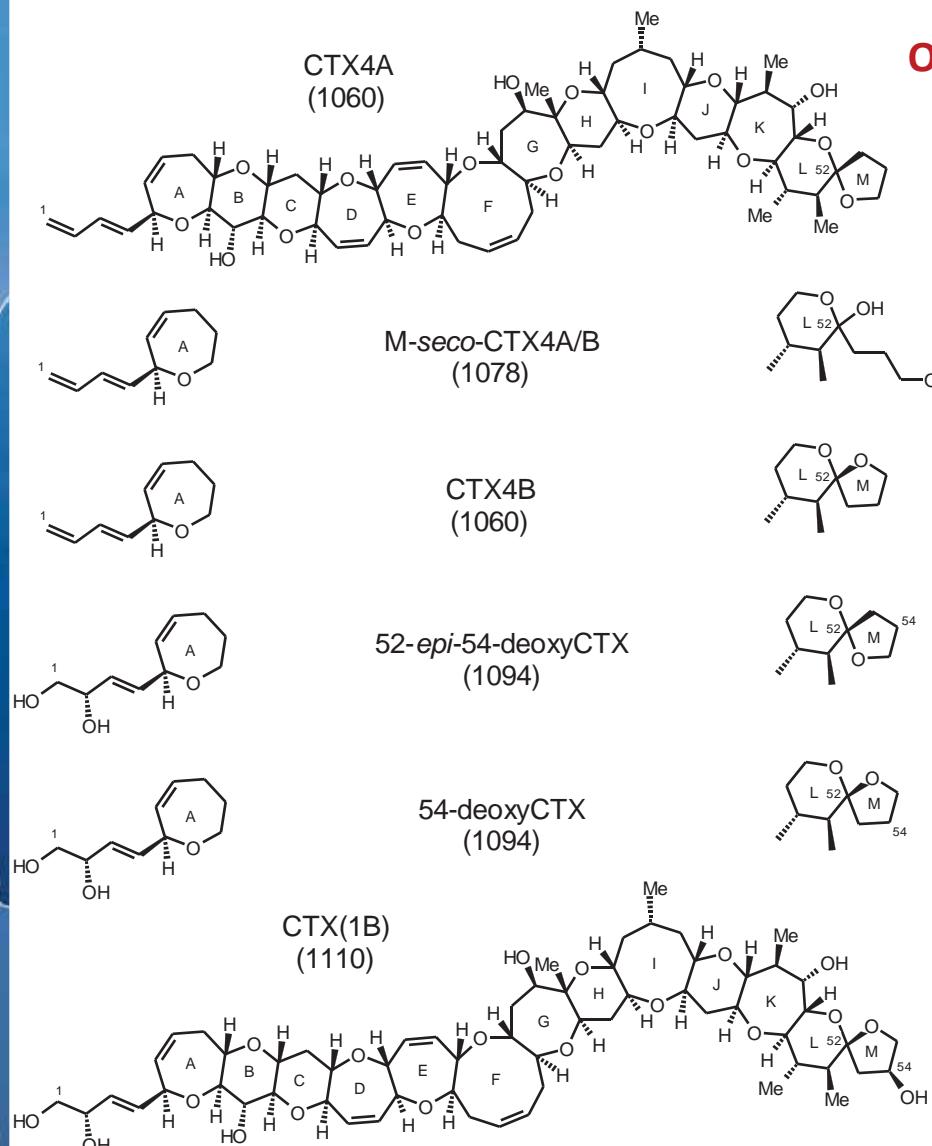


**Gambieroxide**  
(Watanabe et al., 2015)



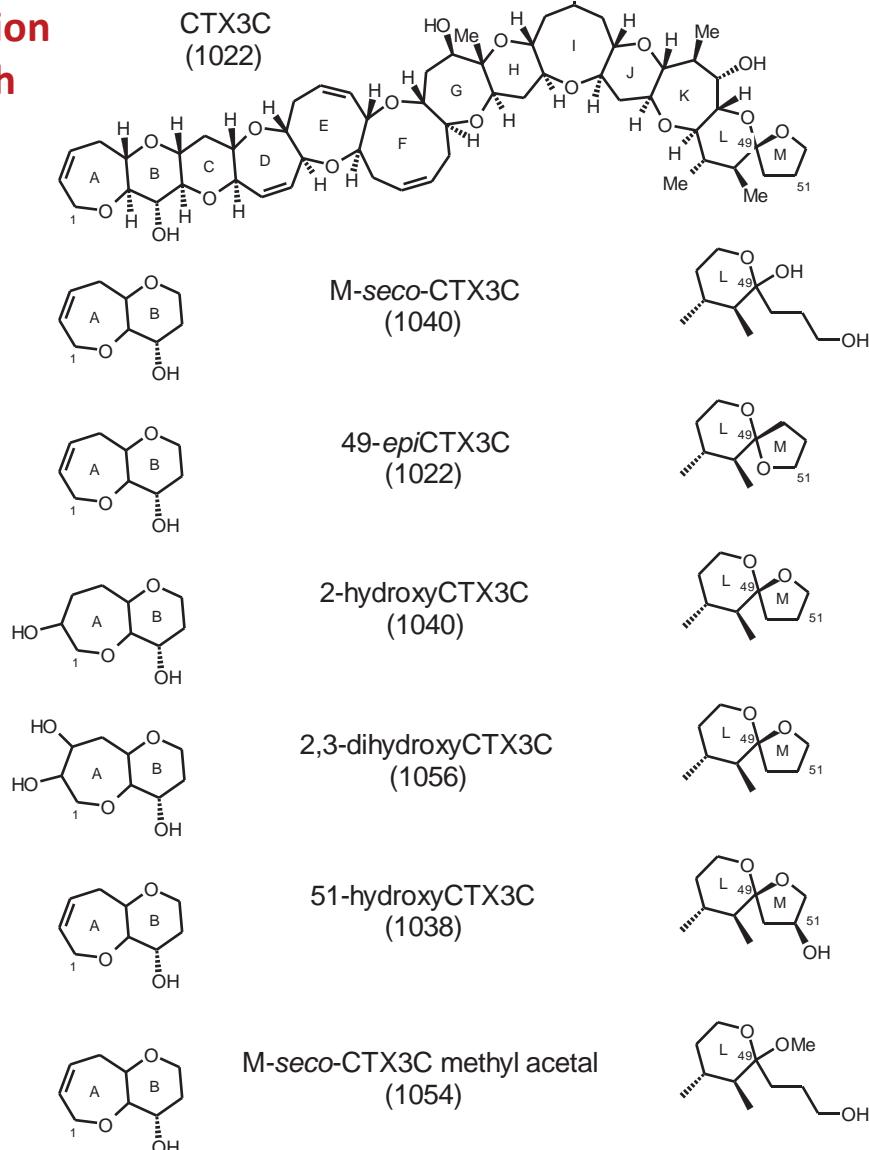
**Ciguatoxin-3C**  
(Satake et al., 1993)

## CTX1B Congeners



Oxidation  
In fish

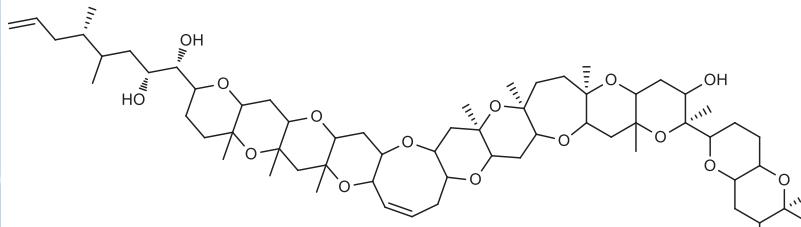
## CTX3C Congeners



Yasumoto, T. Chem. Rec. 2001, 1, 228–242.

# Maïtotoxine

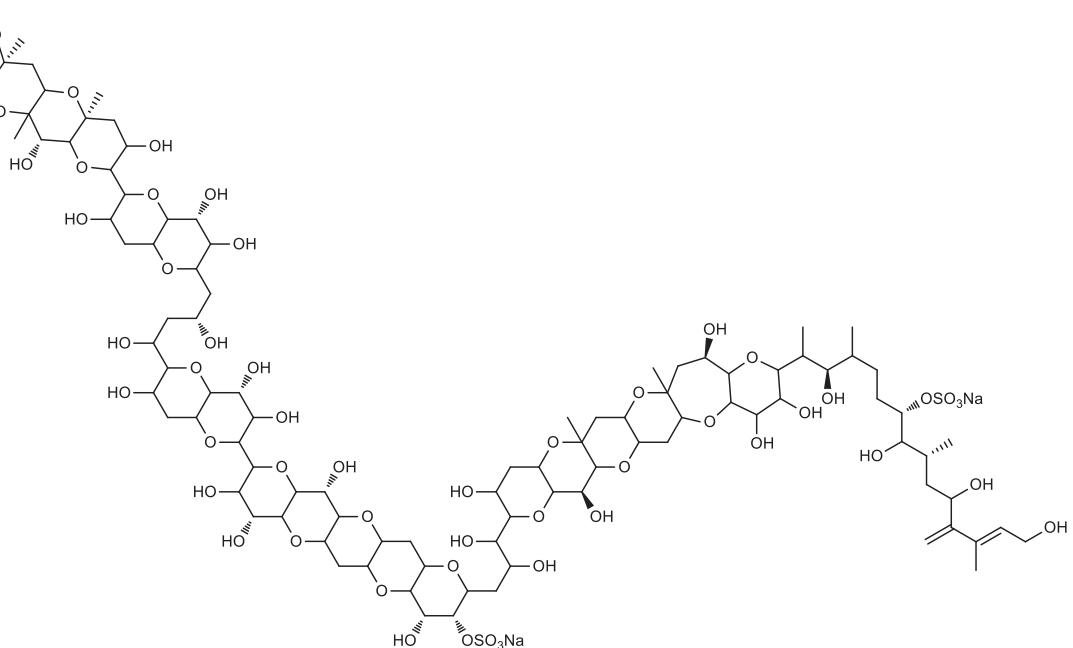
## *Gambierdiscus australis*



Chemical Formula: C<sub>164</sub>H<sub>256</sub>Na<sub>2</sub>O<sub>68</sub>S<sub>2</sub>

Exact Mass: 3423,58108

Molecular Weight: 3425,85618



MTX4 : *G. excentricus* (Pisapia et al., 2017)

MTX5 : *G. belizeanus* (Mazzola et al., 2019)

MTX6 : *Gambierdiscus* ribotype 2, CCMP 1655 (Mazzola et al., 2019)

# Non-targeted LC-HRMS analysis

## *G. excentricus* VG0791

### Liquid chromatography:

Column: Kinetex C<sub>18</sub> (50 x 2.1 mm, 2.6 µm)

Mobile phases: A: H<sub>2</sub>O / B: aq. CH<sub>3</sub>CN (95%)

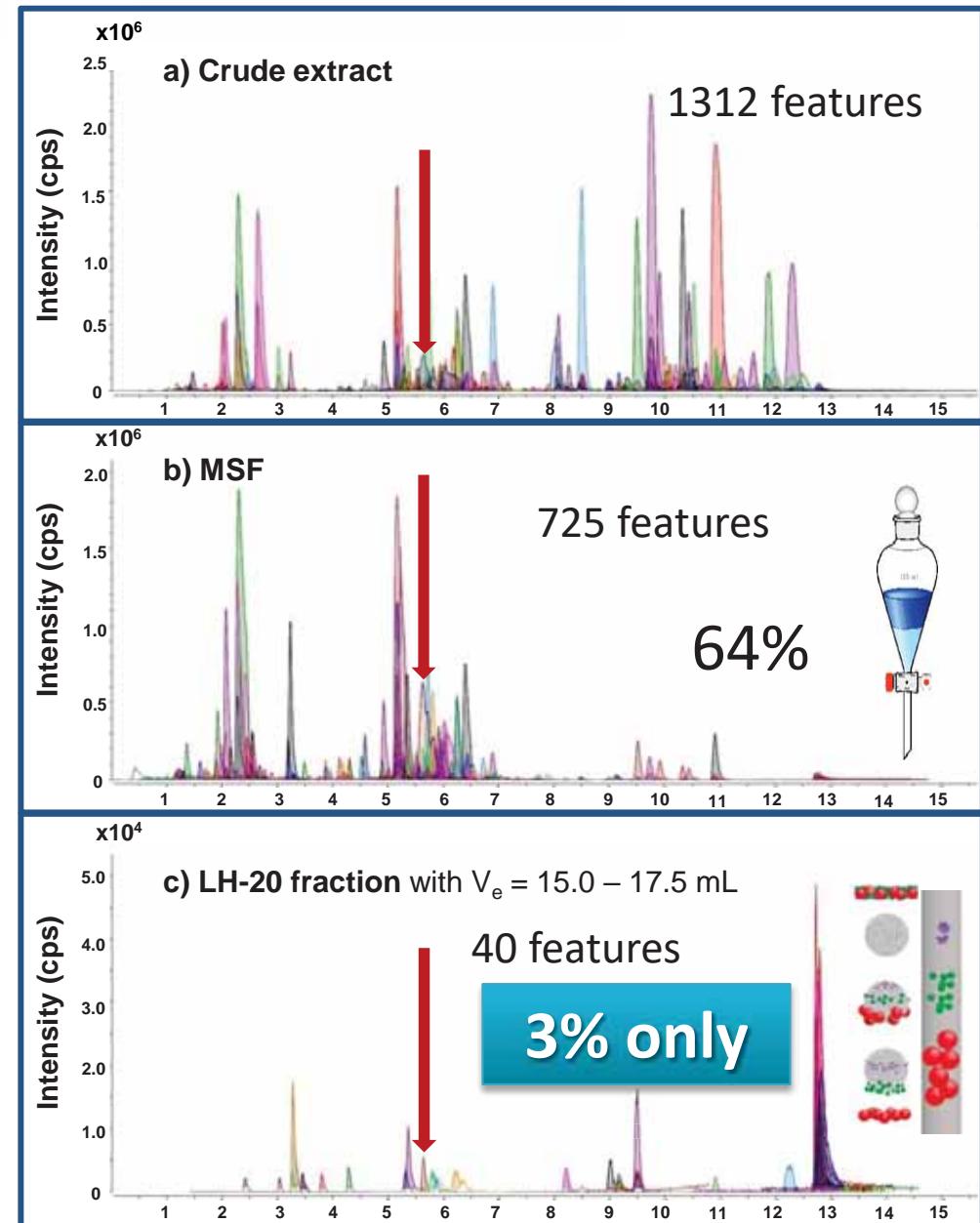
HRMS: Q-Tof 6550 (iFunnel, Agilent)

ESI<sup>-</sup> / Full scan / ECC

- Absence of known MTX
- Pre-purification steps reduced data complexity (1312 → 40 features)
- HRMS permitted evaluation of features for similarity with known MTX

### → Discovery of maitotoxin-4 (MTX4)

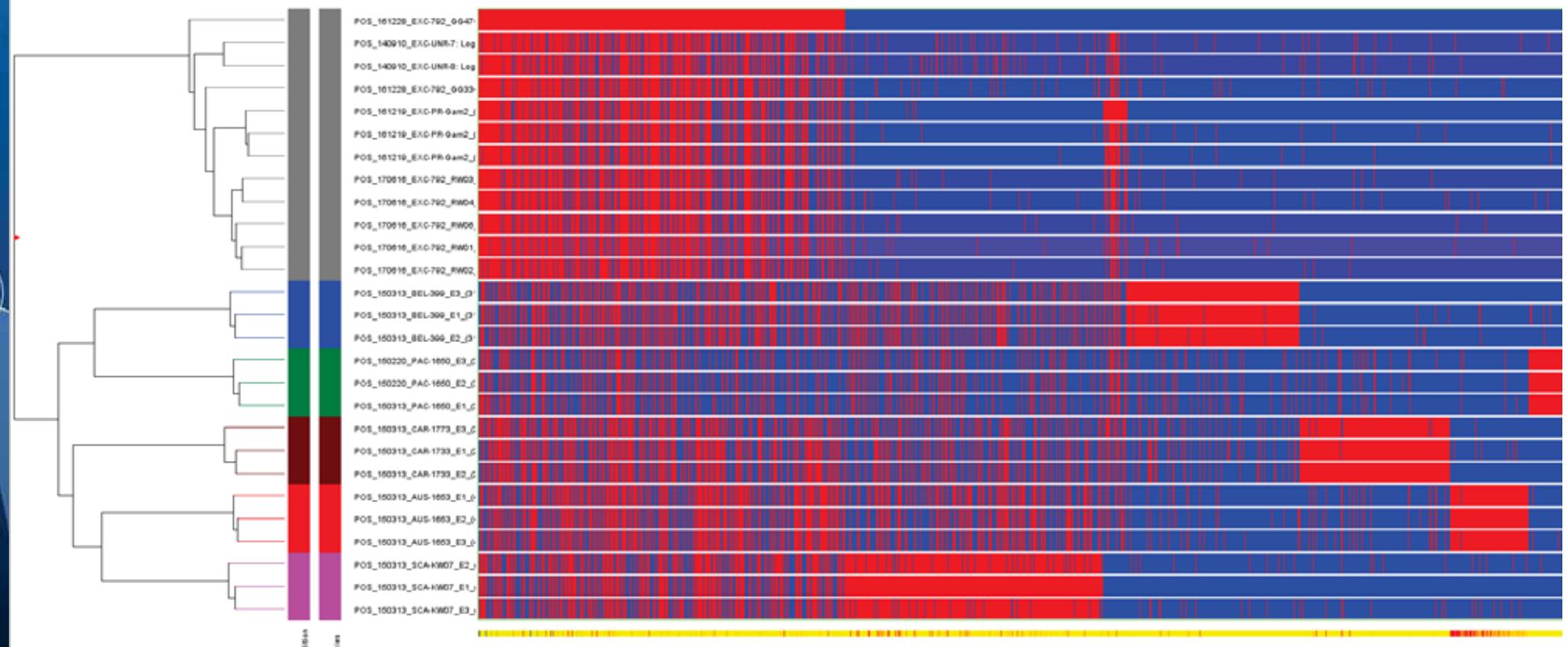
Similar retention time (RT) and similar mass (M) as MTX std



Pisapia et al., 2017 Marine Drugs

# Diversité des métabolites de *Gambierdiscus*

**Hierarchical clustering of 6 *Gambierdiscus* species  
(UPLC – HRMS, ESI<sup>+</sup>)**



# Focus on high masses of 6 *Gambierdiscus* species (3000 – 5000 dalton, UPLC – HRMS, ESI<sup>-</sup>)

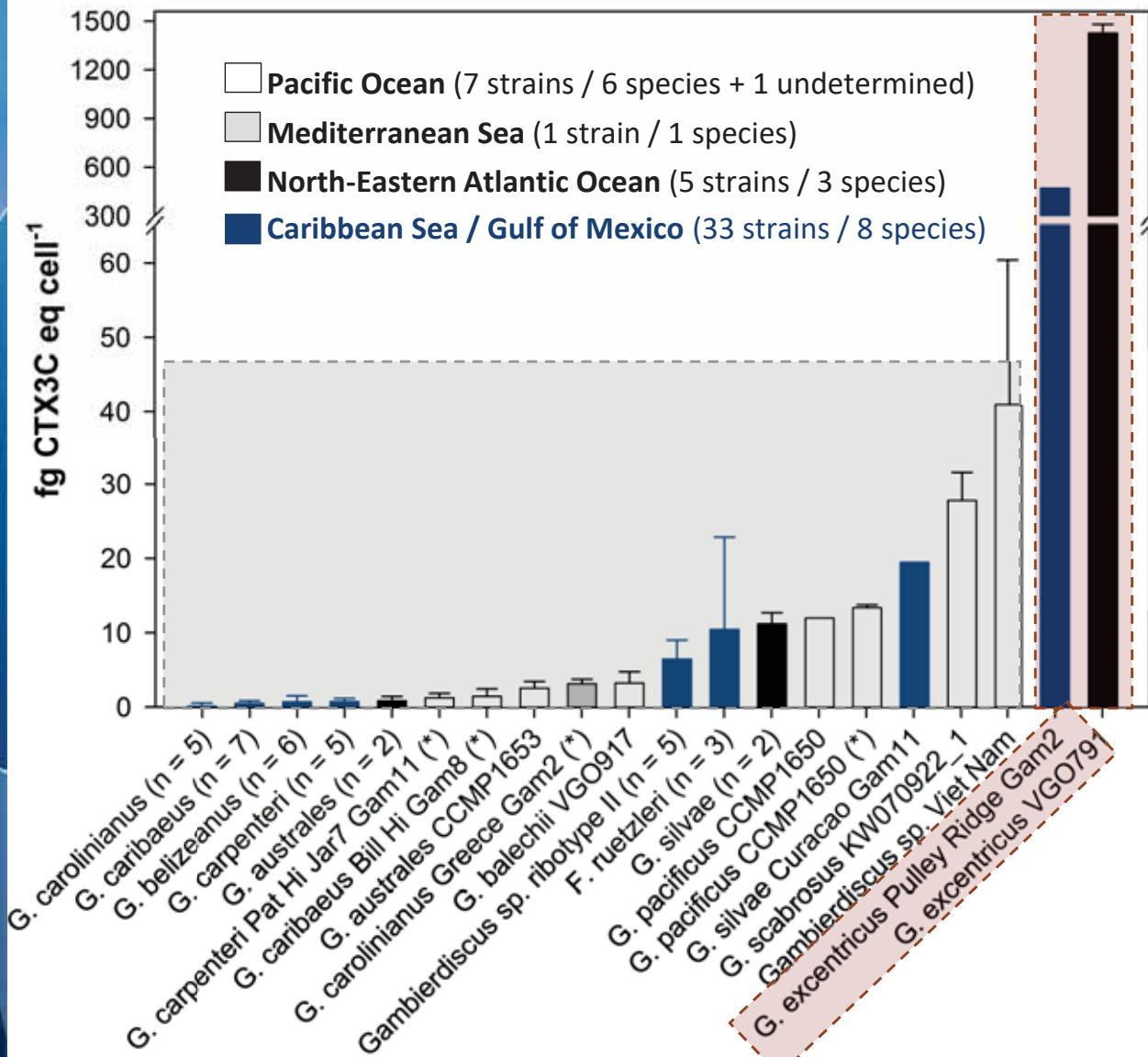
MTX4

Group	Mass	Found	Missed	Height	RT (avg)	AUS	BEL	CAR	PAC	SCA	EXC
3703	3000	3	24	31674	4,602			3			
3152	--	4	23	31358	4,338			3			
362	--	3	24	247145	7,334		3				
1506	3292,482	12	15	33959	4,796						12
2423	--	3	24	32869	7,431		3				
3201	--	3	24	22986	7,624		3				
1951	--	3	24	25304	7,679		3				
2145	--	5	22	45992	4,341	3					
3530	--	3	24	29118	4,361	3					
2167	--	3	24	28085	7,376		3				
5013	--	3	24	24981	5,449						3
1671	--	5	22	35555	7,465		3				
1080	--	3	24	46299	4,372						2
2144	--	4	23	22426	4,429	2					2
4118	--	11	16	28236	4,878		3	3	3		
5747	--	3	24	23012	5,18	3					
1346	--	3	24	150849	5,095	3					
2488	--	3	24	71850	4,907	3					
1921	--	5	22	86702	5,098	3					
2387	--	6	21	29176	5,024	3					3
2130	--	3	24	84733	4,905	3					
3898	--	3	24	32570	4,905	3					
3430	--	3	24	32716	4,905	3					
678	--	11	16	26312	4,066						10
398	--	15	12	26538	4,071		3				12
3030	--	6	21	27432	3,967		3				3
726	--	11	16	19955	2,553						11
2873	--	3	24	59804	2,465		3				
2110	--	5	22	88011	2,478		3				
3861	--	5	22	31595	2,495		3				
4514	5000	5	22	26768	2,472		3				

Mass increasing from 3000 – 5000 da

# Assessment of ciguatoxicity using the N2a assay

Pisapia et al., 2017 (Harmful Algae) & Litaker et al., 2017 (PLoS One)



Inter-species variability:  
 $\approx 8000x$   
 $[0.17 - 1426 \text{ fg CTX3C eq cell}^{-1}]$

*G. excentricus* (Atlantic)  
exhibits particularly high  
CTX-toxicity ( $\text{pg cell}^{-1}$ )

(Fraga et al., 2011)

$\rightarrow \approx G. polynesiensis$  (Pacific)

(Chinain et al., 2010; Munday et al., 2017)

All the other  
species/phylotypes:  
 $< 50 \text{ fg CTX3C eq cell}^{-1}$

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# INCREASED BUSH FIRES & HEAT WAVES

Victoria Feb. 2009  
300 people killed  
7,000 homeless



# SYDNEY DUST STORM 23 Sept 2009: 16 Tg



**IPCC**

INTERGOVERNMENTAL  
PANEL ON  
CLIMATE CHANGE



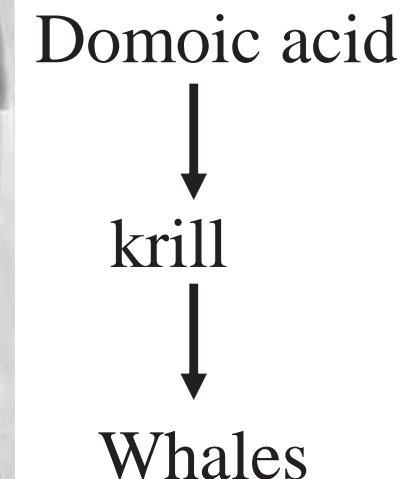
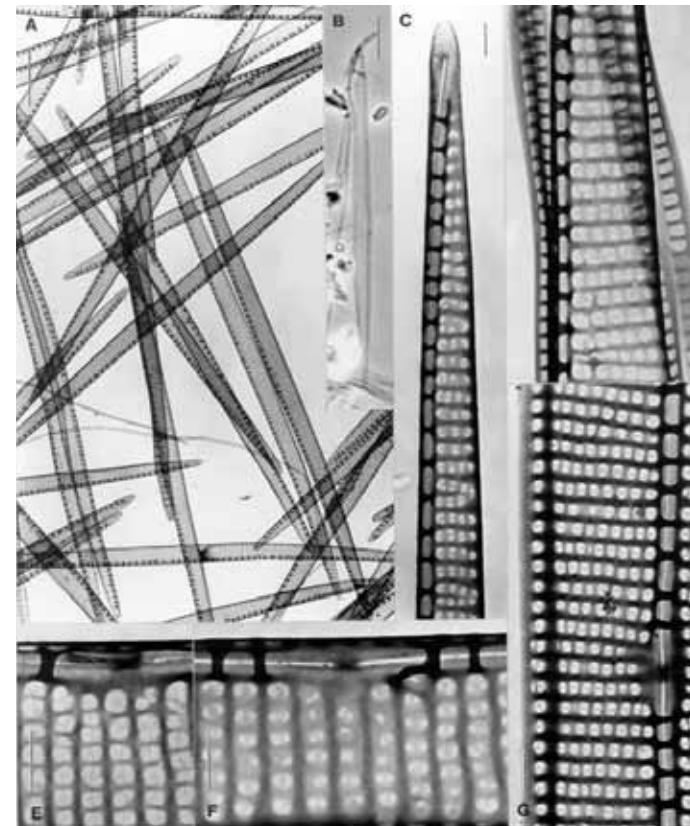
Courtesy: Gustaaf Hallegraeff

# Ouverture du passage Nord-Ouest



Melting Arctic sea-ice returned diatom *Neodenticula seminae* from Pacific to Atlantic first time since 800.000yrs  
(Reid 2007, Glob.Change Biol.)

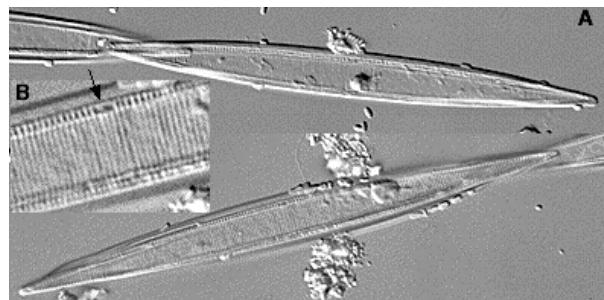
Courtesy: Gustaaf Hallegraeff



# Xynthia &

Xynthia : une tempête  
frappe de manière  
inattendue la côte  
atlantique le 27-28/2/2010

*Pseudo-nitzschia*, le  
producteur d'acide  
domoïque fait une  
efflorescence 50-100 fois >  
qu'avant



# *Pseudo-nitzschia*

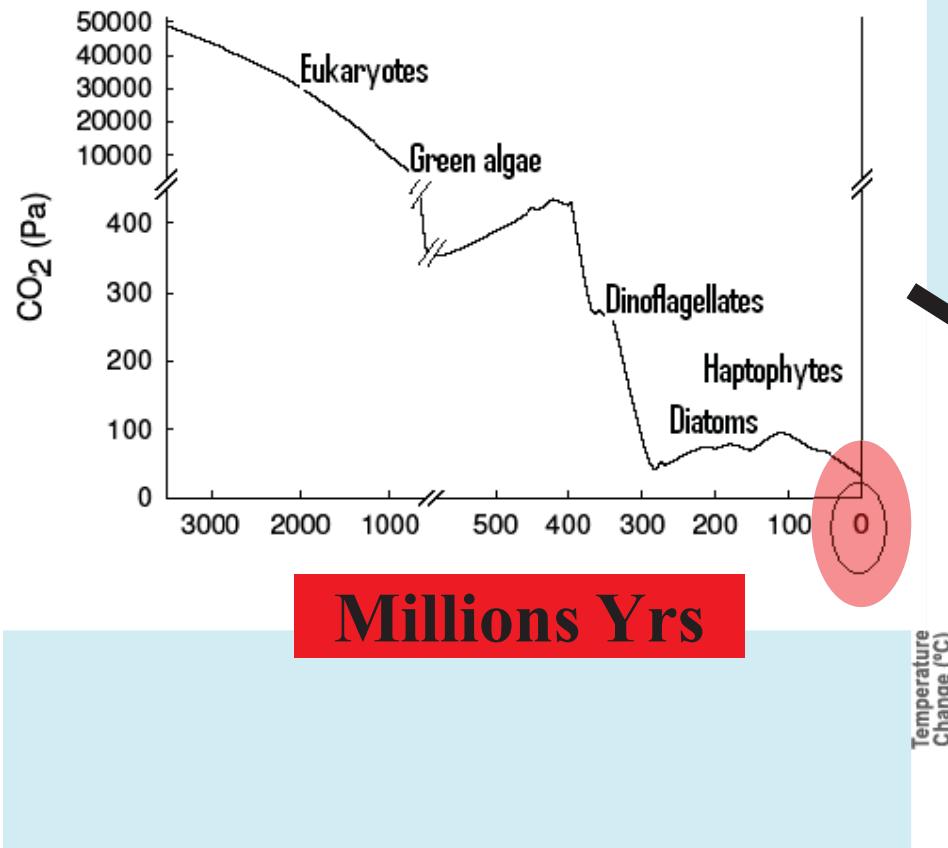
Xynthia,  
PLACE PUBLIQUE  
une tragédie prévisible

57 people died and hundreds put out of their homes

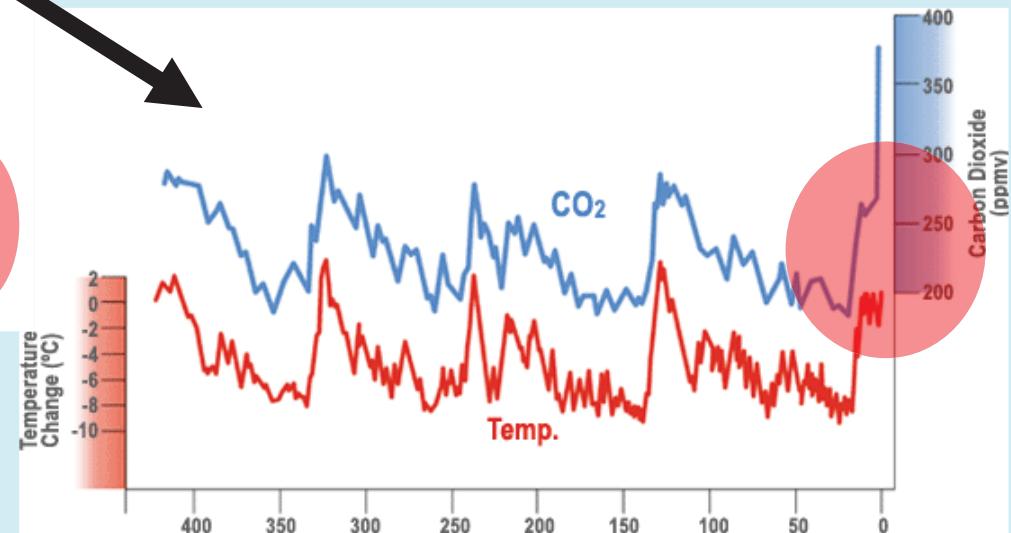


2 années de fermeture de la pêche !

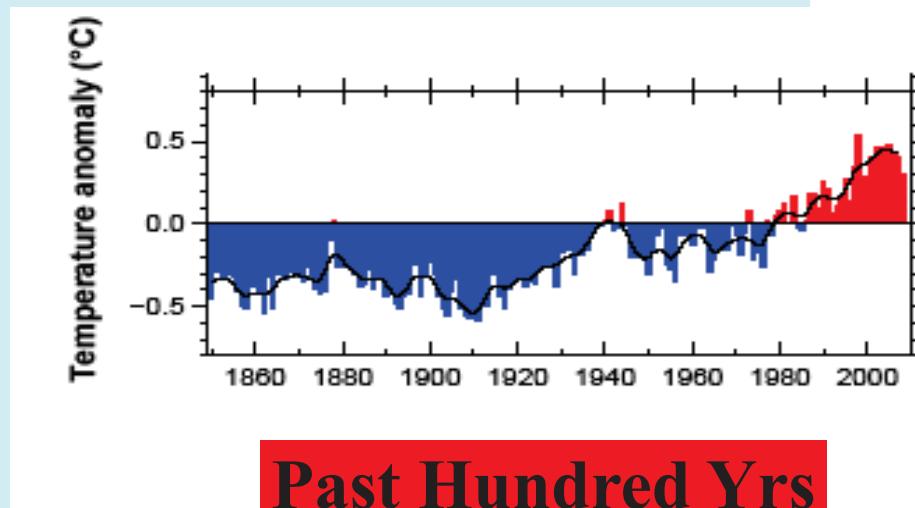
Cyanobacteria



## Climate Change is a matter of scale and time



Thousands Yrs



Past Hundred Yrs

Courtesy: Gustaaf Hallegraeff

# Lotterie : les gagnants et les perdants

- Increased temperature
- Enhanced surface stratification
- Alteration of ocean currents/nutrient upwelling
- Stimulation of photosynthesis by elevated CO<sub>2</sub>
- Ocean acidification (“the other CO<sub>2</sub> problem”)
- Heavy precipitation/ storm events

Complex factor interactions /feedback mechanisms exists  
Laboratory simulations rarely allow for sufficient acclimation

## **Phenotypic consequences of 1,000 generations of selection at elevated CO<sub>2</sub> in a green alga**

**Sinéad Collins & Graham Bell**

Organism responses will be species- or even strain-specific

**There will be winners and losers!**

Courtesy: Gustaaf Hallegraeff



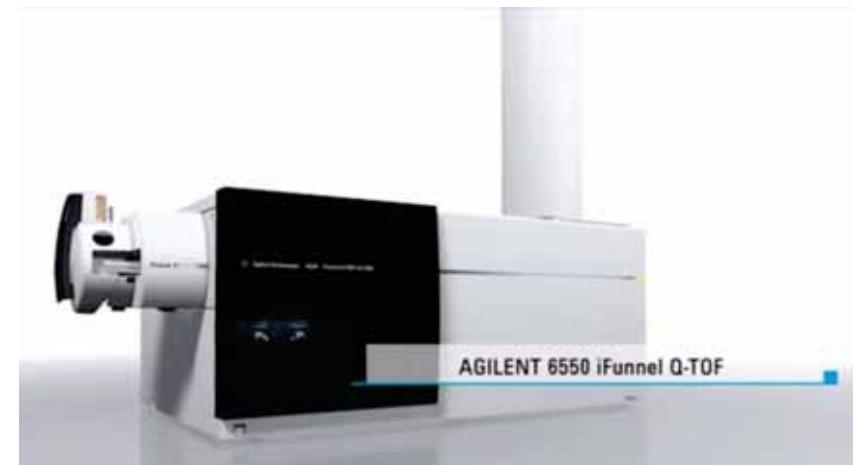
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# Instrumentation for metabolomics



Agilent 1100 series (HPLC) coupled  
to **API4000 (AB-Sciex)**



Agilent 1290 Infinity II (UHPLC)  
coupled to **i-funnel QToF 6550**



ThermoScientific Accela  
(UHPLC) coupled to **Exactive**

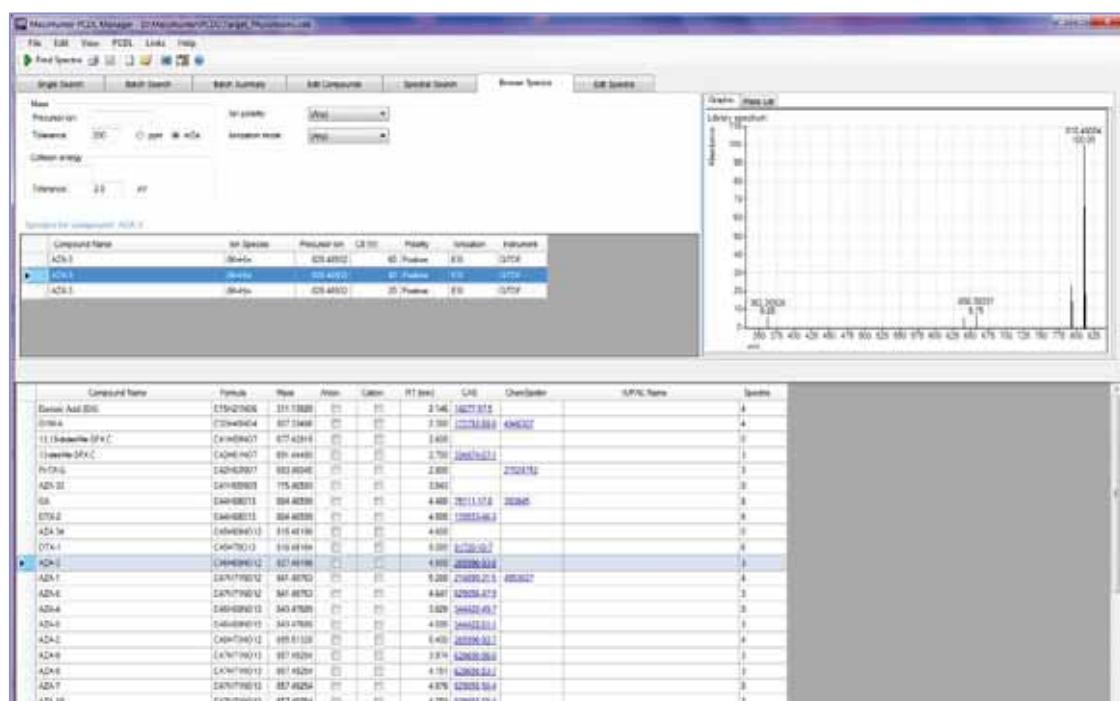
[Zendong et al. \(2015\) \*J. Chromatogr. A\*, 1416, 10-21.](#)

# Base de données (> 500 Toxines)

Toxin family	Toxin group	Number Compound	Molecular structure
ASP	DA	10	10
DSP	OA+DTX	60	13
	AZAs	40	32
	PTXs	17	15
	YTXs	31	16
PSP	STXs	64	60
	TTXs	21	21
FAT	GYMs	7	5
	SPXs	11	11
	PnTXs+PterTXs	12	11
	Imine cyclic	7	6
NSP	PLTXs	11	4
	MTX	1	1
	PbTXs (BTX)	17	12
	Goniodomin A	1	1
	KBTX	10	4
	Tamulamide	2	2
	Gambiertoxins	7	6
	P-CTXs	27	27
	C-CTXs	2	2
	Oscillatoxin	11	11
	Anatoxin	12	12
	Cylindrospermocin	3	3
CYANO	Microcystins	14	14
	Aplysiatoxins	6	6
	Curacins	4	4
	Jamaicamides	3	3
	Antillatoxins	2	2
	Nodularin	7	7
	Kakitoxin	1	1
	BMAA	1	1
	Trichotoxin	1	1
	Karlotoxins	6	3
	Calyculins	14	14
	Gliotoxins	2	2
OTHERS	Polycavernosides	5	5
	Amphidinols	12	12
	Amphidinolides	39	39
	portimine	1	1
	Nakijquinone	4	1
	precursor C-CTXs	4	/

The *PCDL library* currently contains information for over 500 toxins with 400 structures and spectra for approximately 70 compounds (including all regulated toxins in the US and Europe). The library was constructed from existing databases, literature and from spectra acquired on the 6540 and 6550 Agilent Q-ToF instruments.

One of the main inconveniences of non-targeted HRMS data is the large size of raw-data files and time required for data handling and reprocessing. The MassHunter PCDL manager is a module in the MassHunter software suite that allows both for easy visualisation of compounds, spectra and structures in a library collection and for rapid interfacing with other Agilent software modules, i.e. rapid data processing. For instance, a data file from 10 min All-ion HRMS acquisition only requires a few seconds for dereplication against this library.



# Limites de detection (toxins réglementées)

<i>Toxin</i>	<u>Mussel (<math>\mu\text{g/kg}</math>)</u>		
	<i>QqQ</i>	<i>QToF</i>	<i>Exactive</i>
<b><i>PTX2</i></b>	1.00	1.66	1.2
<b><i>PnTX-E</i></b>	0.46	0.47	0.09
<b><i>DA</i></b>	28.69	25.25	24.14
<b><i>AZA1</i></b>	1.36	0.4	0.2
<b><i>AZA2</i></b>	2.14	1.07	2.38
<b><i>AZA3</i></b>	0.45	0.52	0.64
<b><i>OA</i></b>	14.28	1.67	5.57
<b><i>DTX1</i></b>	<b>1.94</b>	<b>3.29</b>	<b>4.91</b>
<b><i>DTX2</i></b>	1.95	1.94	3.02
<b><i>YTX</i></b>	<b>12.4</b>	<b>21.52</b>	<b>33.45</b>
<b><i>homo-YTX</i></b>	11.77	17.43	5.39
<b><i>13-desmeSPX-C</i></b>	4.13	0.15	0.3
<b><i>GYM-A</i></b>	1.61	0.9	0.87
<b><i>PnTX-F</i></b>	<b>0.07</b>	<b>0.31</b>	<b>0.31</b>
<b><i>PnTX-G</i></b>	0.22	0.09	0.29

- Bonnes LoDs pour les trois analyseurs par rapport à la limite légale
- LoDs de l'HRMS comparable ou plus bas comparé à LRMS pour la plupart des toxins sauf **YTX, PnTX-F, DTX1** Zendong et al. (2015) *J. Chromatogr. A*, 1416, 10-21.

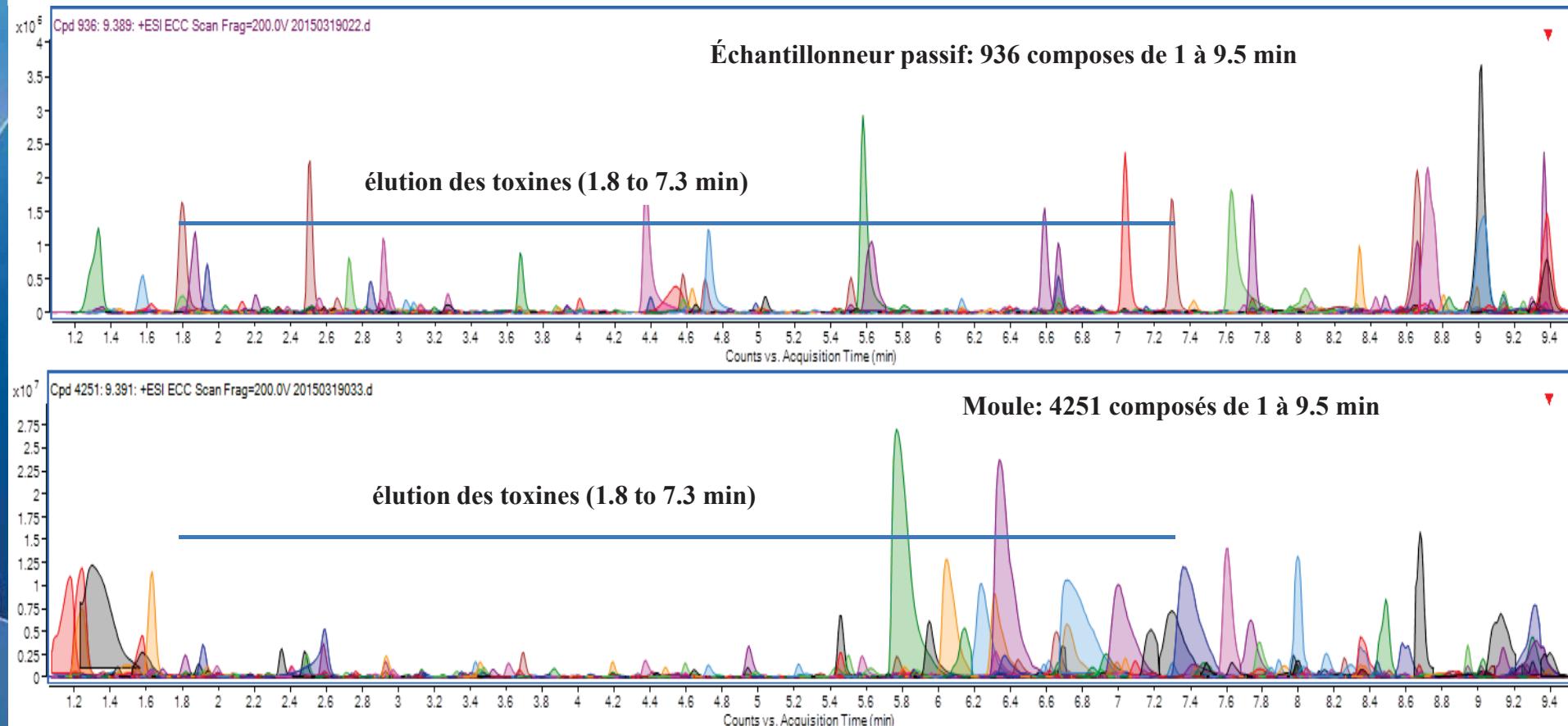
# HRMS: analyse non-ciblée

## échantillonnage passif vs. matrice complexe



i-funnel QToF 6550

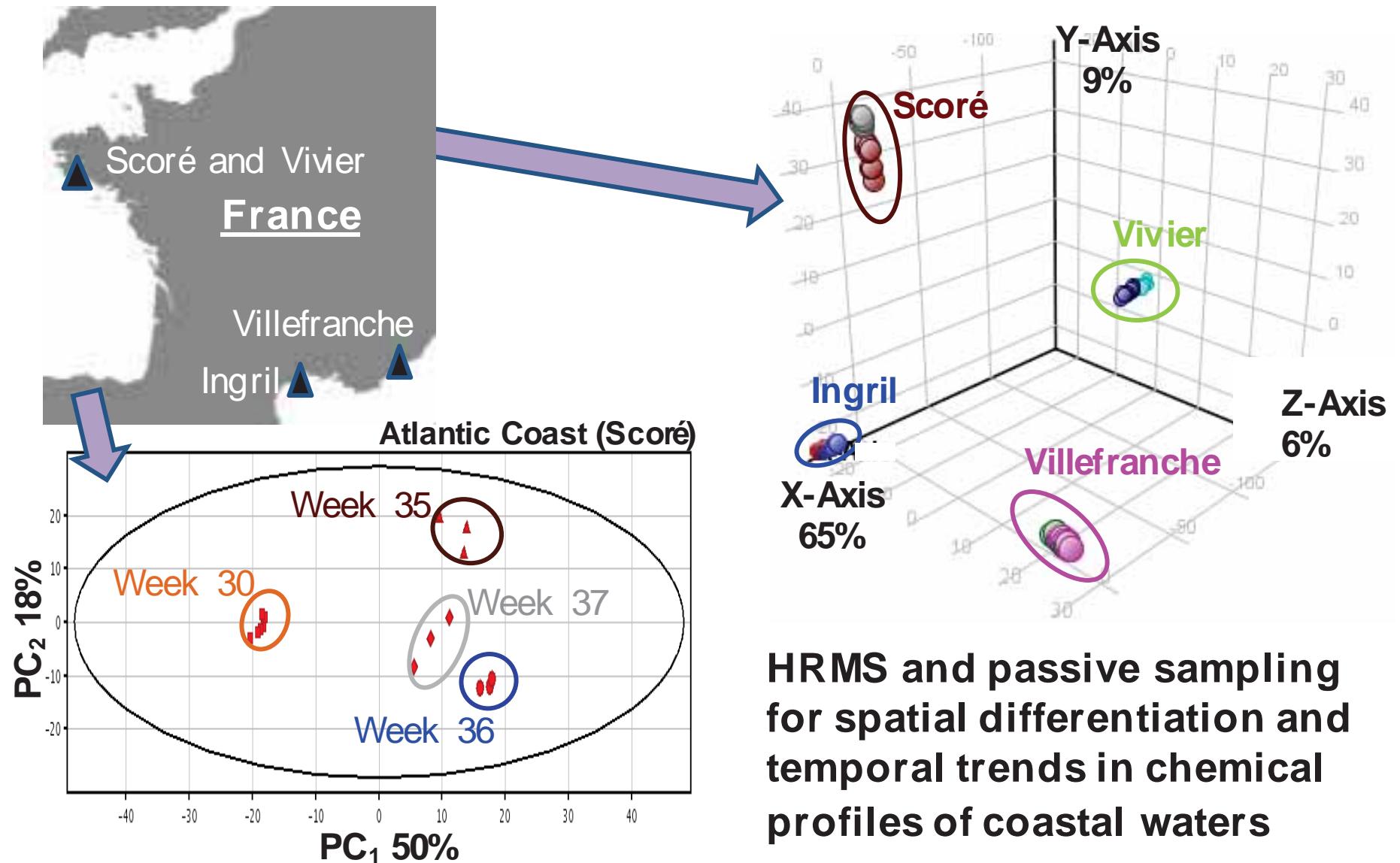
Zendong et al. (2015) *J. Chromatogr. A*, 1416, 10-21.

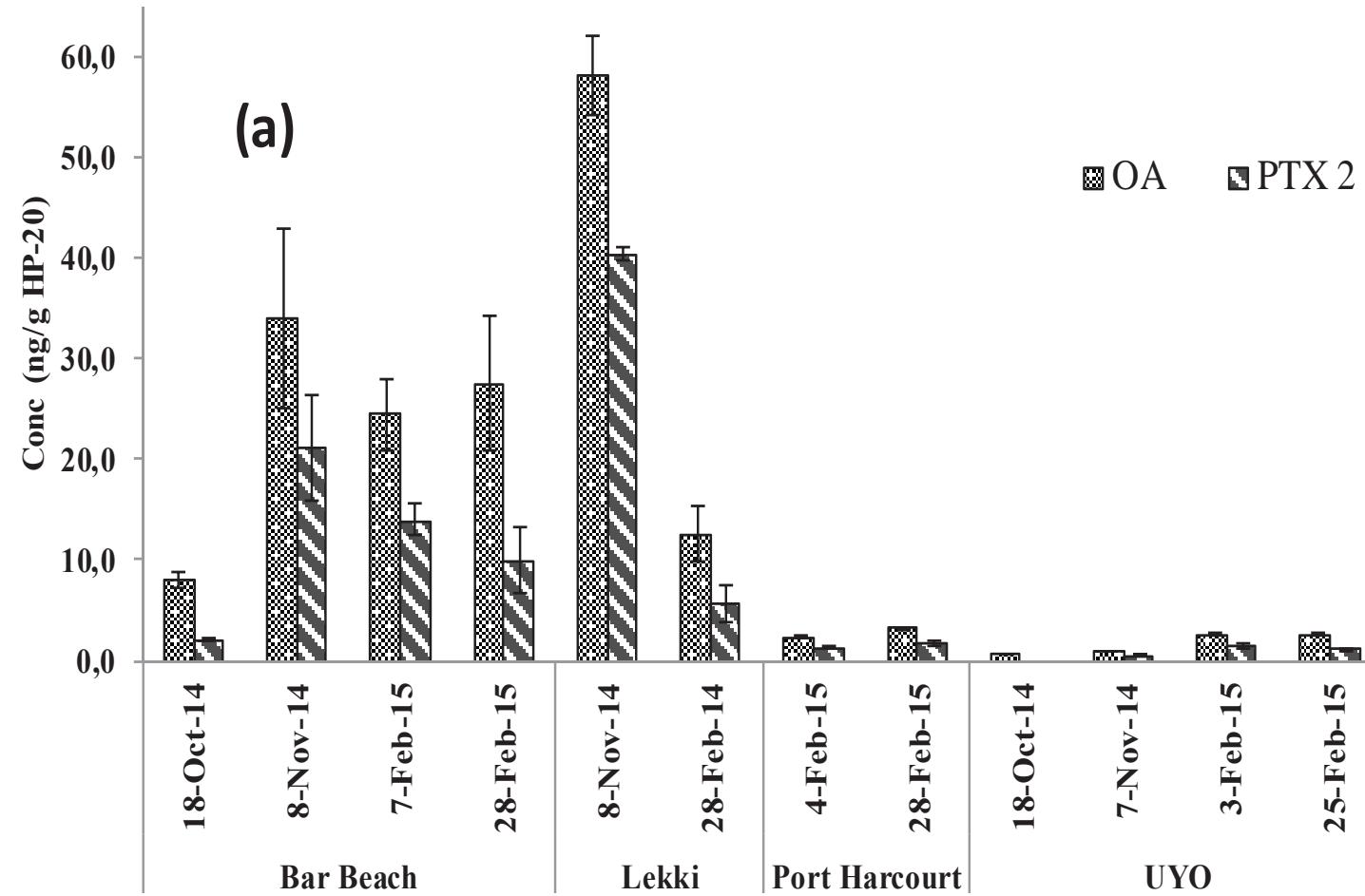


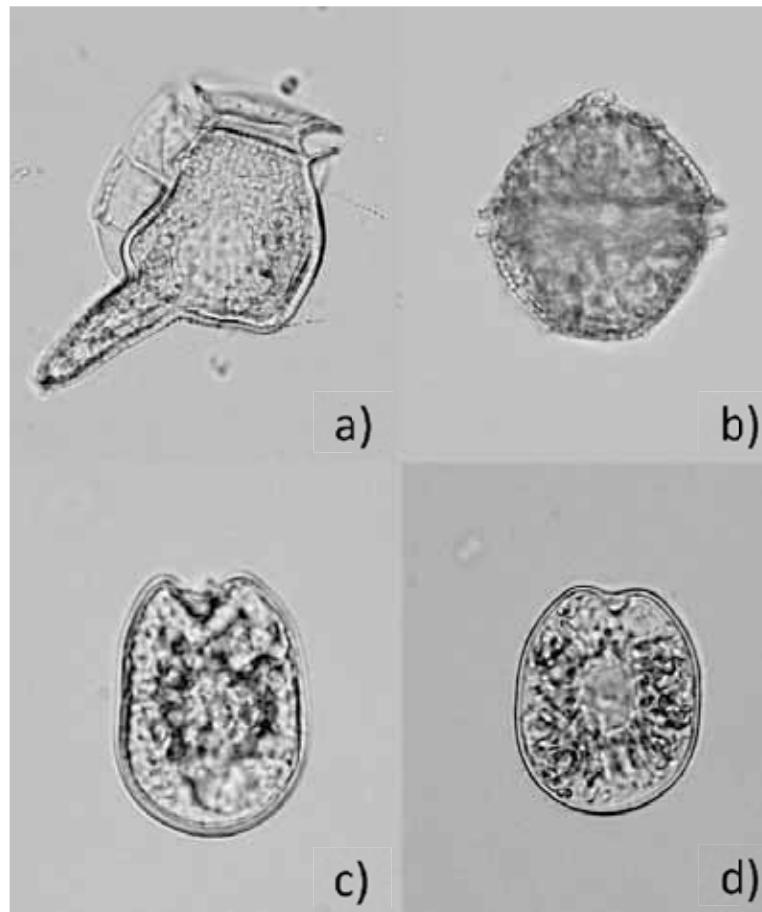
- Image of many, many compounds present in each sample
- Retrospective analysis of acquired data for discovery of new or emerging toxins

# échantillonnage passif + HRMS, France 2014

Zendong et al. (2016) *Environ. Sci. Technol.* 15 (16) 8522-8529.



Découverte de toxines d'algues dans des eaux Nigérianes (Océan Atlantique) 2014/5Zendong et al. (2016) *Toxicon*, 114, 16-27.

Découverte de toxines d'algues dans des eaux Nigérianes (Océan Atlantique) 2014/5Zendong et al. (2016) *Toxicon*, 114, 16-27.

Marine dinoflagellates identified on Bar Beach (Lagos State, Nigeria, 21 February 2015): a) *Dinophysis caudata* ( $L = 100 \mu\text{m}$ ), b) *Lingulodinium polyedrum* ( $L \times W: 40 \times 38 \mu\text{m}$ ), c) *Prorocentrum* sp1 ( $L \times W : 37 \times 27 \mu\text{m}$ ) and d) *Prorocentrum* sp2 ( $L \times W: 36.3 \times 28.8 \mu\text{m}$ ).

# Perspectives

- Toxines potentiellement léthales pour l'Homme mais sont aussi composés modèles pour le traitement des maladies
- Rôle écologique loin de la toxicité chez l'Homme ?
- Diversité chimique naturelle peut servir comme inspiration pour la chimie médicinale (QSAR...), plusieurs genres de dinoflagellés montrent une large diversité chimique (génome complexe)
- Changement Climatique suggère vigilance accrue pour garantir la santé publique, l'analyse non-ciblée peut aider

Merci pour votre attention

