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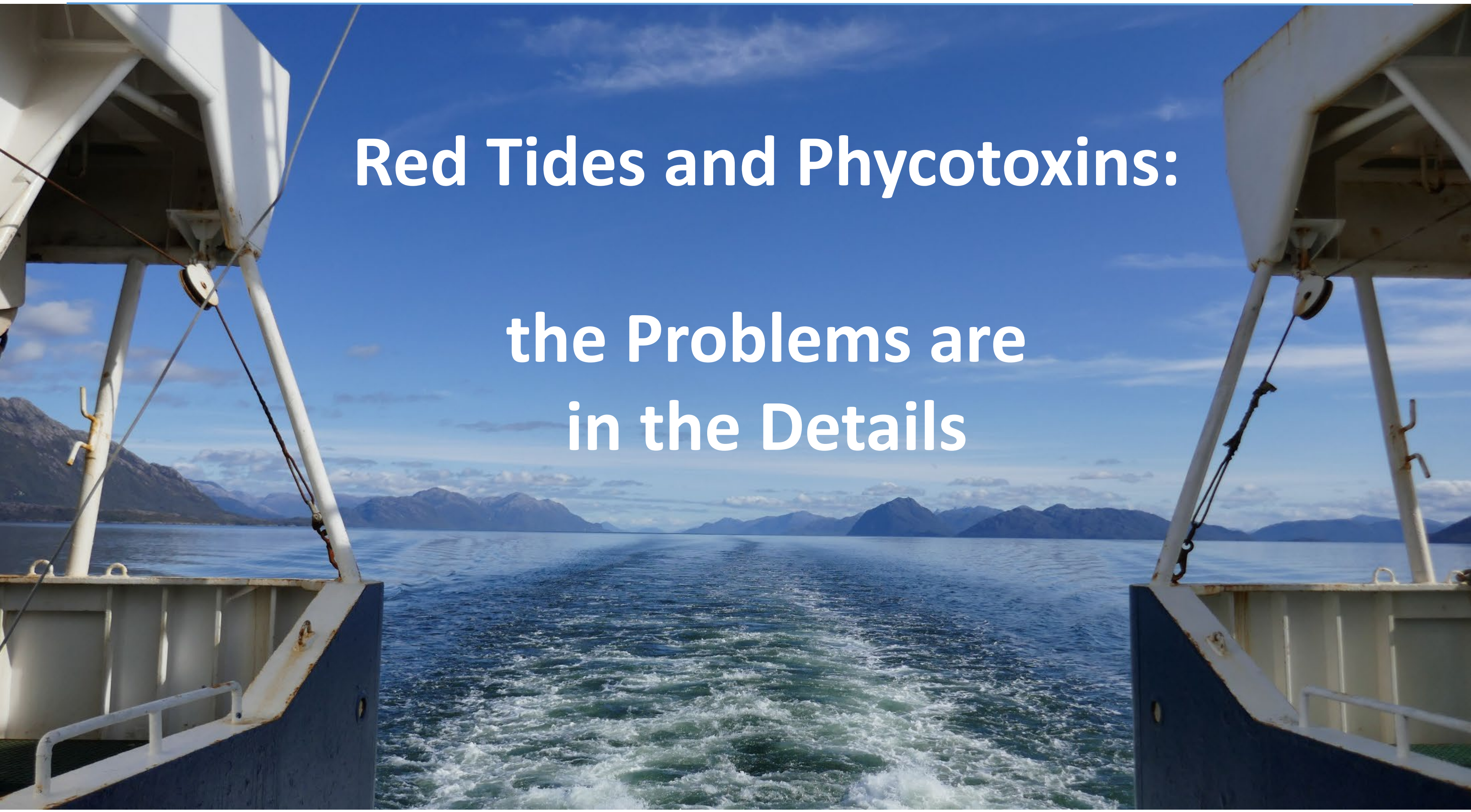
Red Tides and Phycotoxins:

the Problems are in the Details



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XLI Congreso Chileno de Ciencias del Mar, UCSC, Concepción, 23-27 Mayo 2022, B. Krock

Introduction

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Chile is prone to HAB events, e.g. 2016

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Introduction

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February/March 2016, Regíon de Los Lagos: massive bloom of *Pseudochattonella* cf. *verruculosa*


https://www.mundoacuicola.cl/new/mortalidad-de-salmones-por-fans-no-aceptemos-los-mismos-errores/

Informe Final, Comisión Marea Roja
https://www.economia.gob.cl/wp-content/uploads/2016/11/InfoFinal_ComisionMareaRoja_24Nov2016-1.compressed.pdf

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Introduction

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April/May 2016, Región de Los Lagos: massive bloom event of *Alexandrium catenella*

Informe Final, Comisión Marea Roja
https://www.economia.gob.cl/wp-content/uploads/2016/11/InfoFinal_ComisionMareaRoja_24Nov2016-1.compressed.pdf

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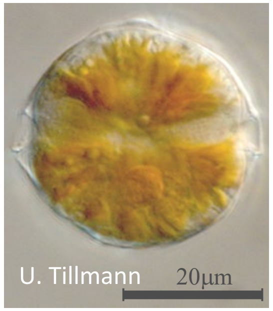
Introduction

Both blooms were almost coinciding in time and space and caused mass mortality of marine life, especially in salmon aquaculture

Mechanistically both blooms were differrent:

Pseudochattonella cf. *verriculosa*:
Ichthyotoxic

Alexandrium catenella:
PSP Toxin producer



Ichthyotoxins

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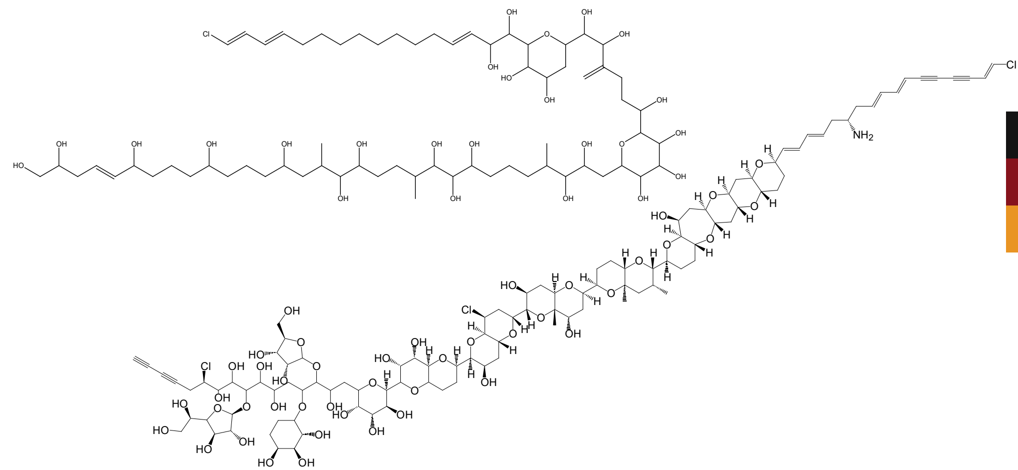
Variability

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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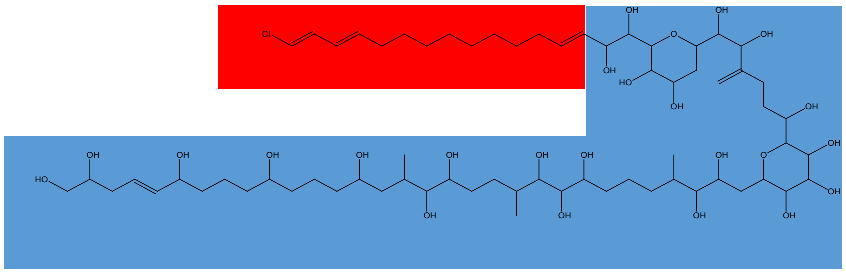
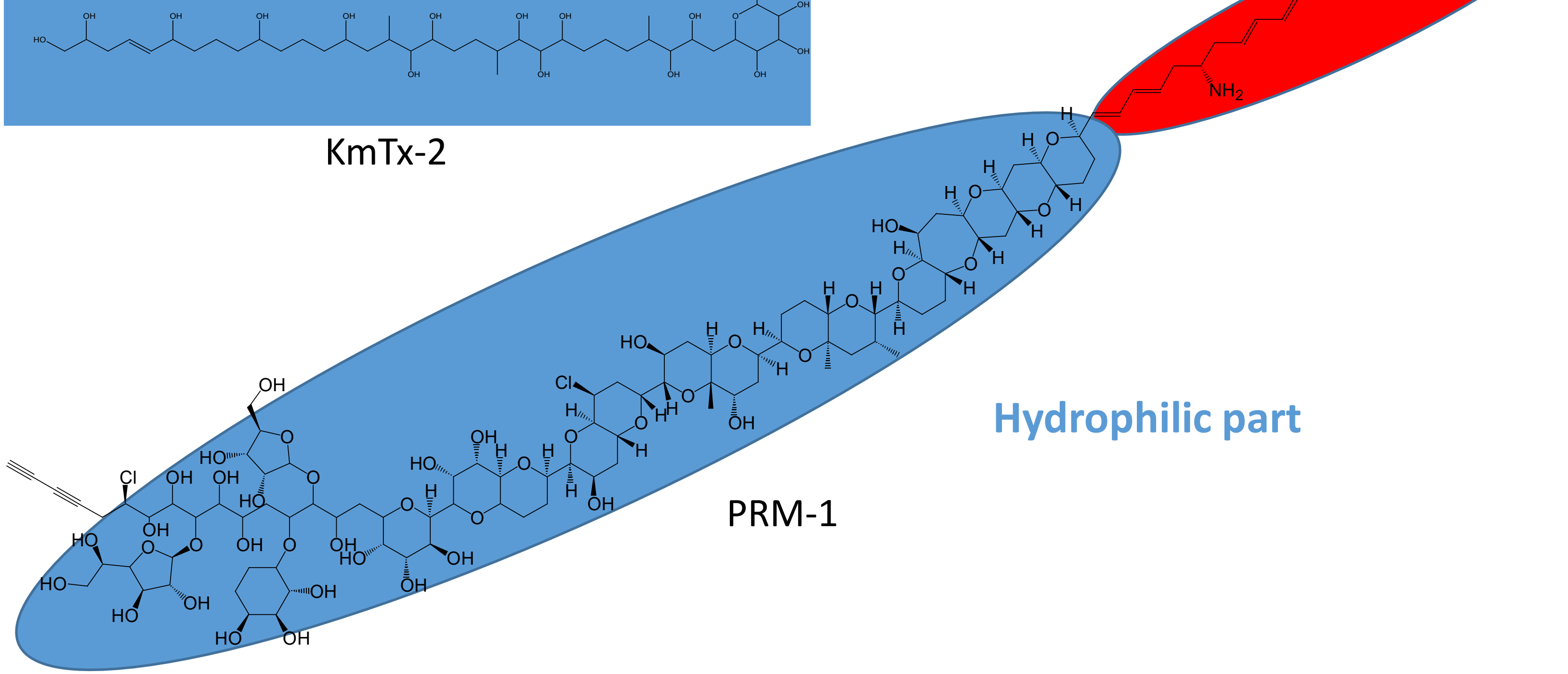
Ichthyotoxins

Known Ichthyotoxins



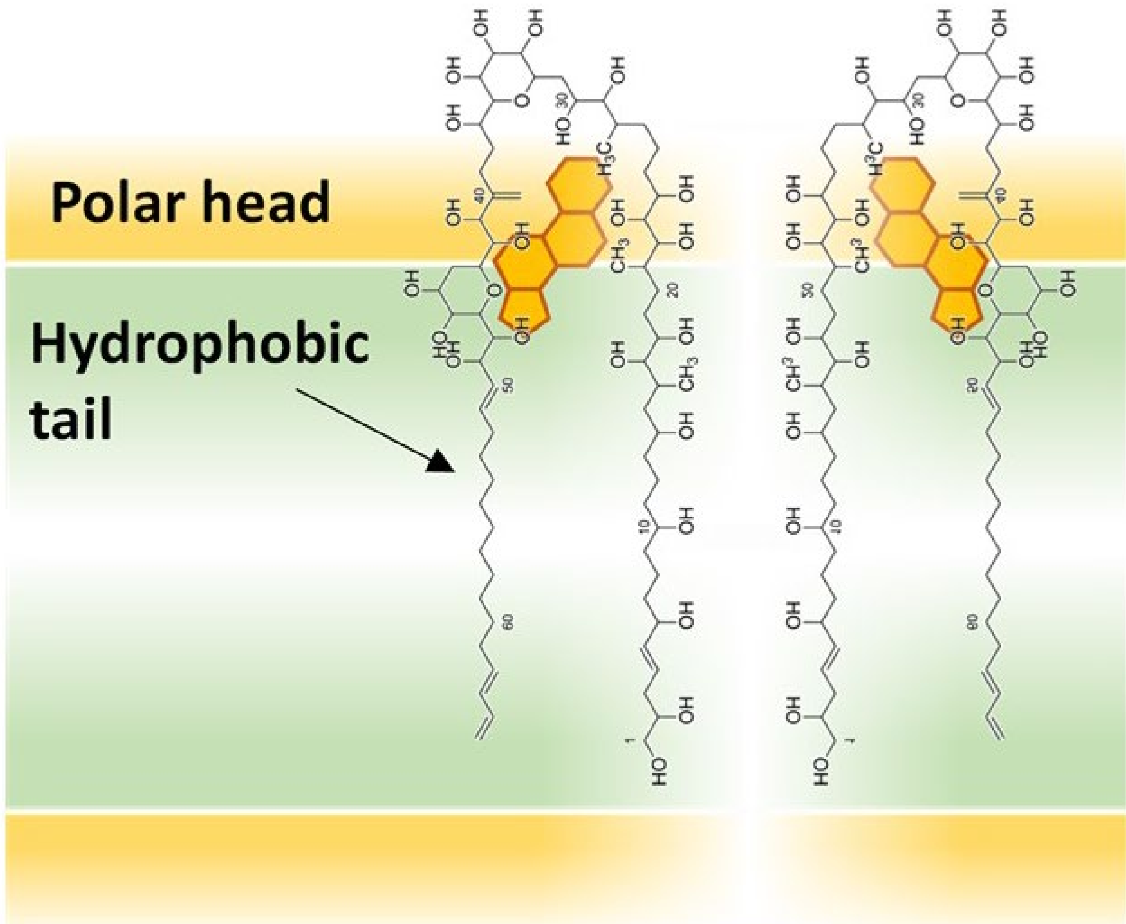
KmTx-2



PRM-1

Ichthyotoxins

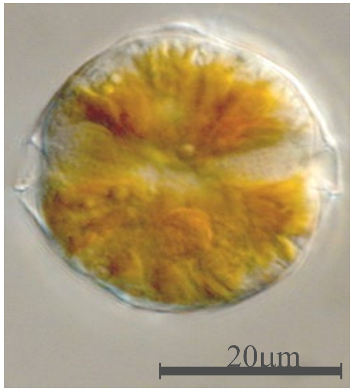
Mode of action



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

Ichthyotoxins

Lytic Effect



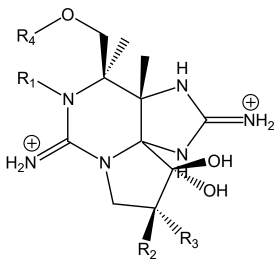
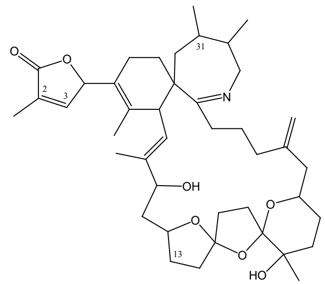
Alexandrium catenella
strain 2 (Alex2)

Rhodomonas salina

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

Video: U. Tillmann

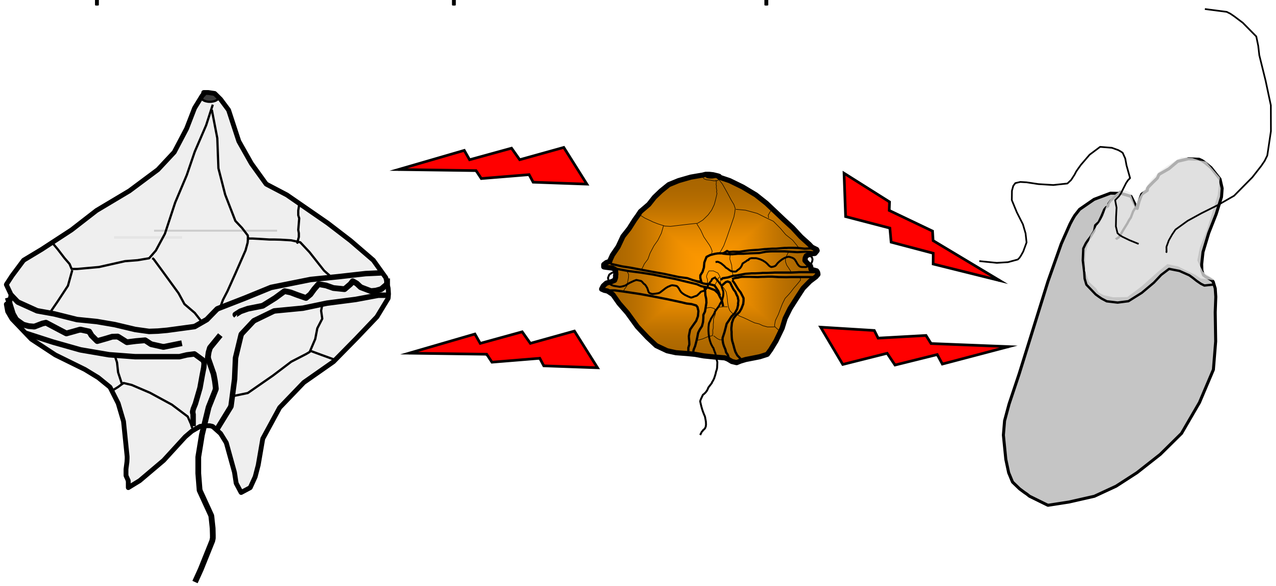
Ichthyotoxins

Organism	Chemical compound		Ecological Function
	PSP-Toxins	Spirolides	
Alexandrium spp.			?
Pseudochattonella cf. *verriculosa* (*Alexandrium* spp.)	?		Defense against Predators Elimination of Competitors

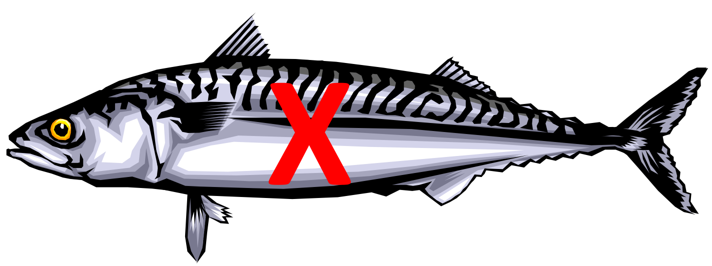
Ichthyotoxins

Current Hypothesis:

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



Ichthyotoxicity is a collateral damage of protistan allelochemistry



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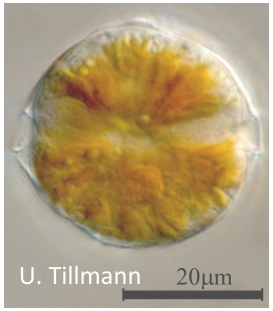

Variability of phycotoxins

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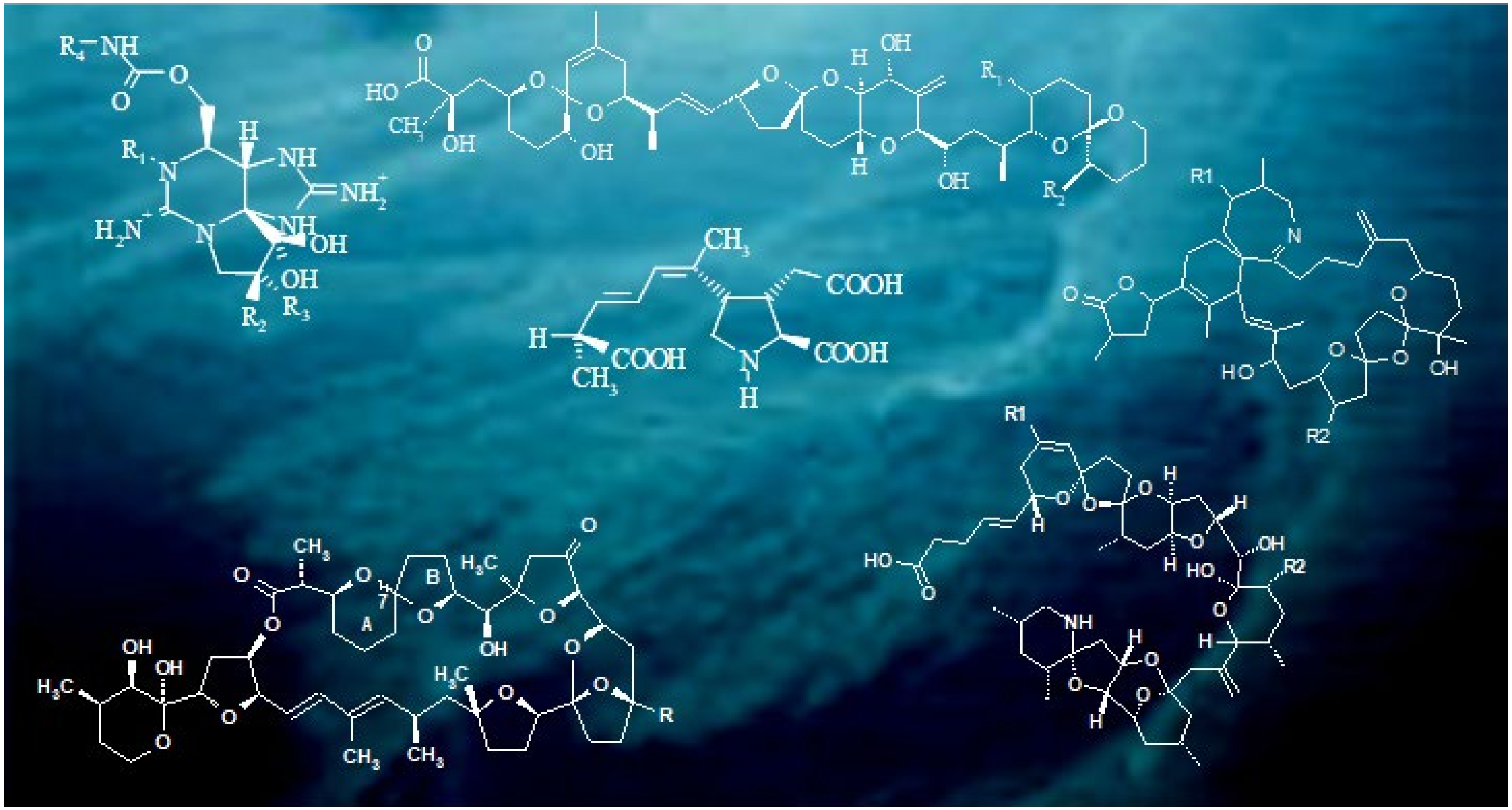
High variability of phycotoxin casses



Need of monitoring for seafood safety

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Variability of phycotoxins

Mouse Bioassay (MBA)

Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

MBA is banned in the United States and the European Union

Variability of phycotoxins

Problems of MBA:

- Poor reproducibility
- Low sensivity
- Low specifity
- Ethical concerns

Problems of LC-MS/MS:

- High cost
- Need of trained staff
- **Only targeted analysis**

Variability of phycotoxins

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Toxin group	Analogue	TEF
OA-group toxins (OA-equivalents)	OA	1
	DTX1	1
	DTX2	0.6
AZA-group toxins (AZA-equivalents)	AZA1	1
	AZA2	1.8
	AZA3	1.4
YTX-group toxins (YTX-equivalents)	YTX	1
	1a-homoYTX	1
	45-hydroxyYTX	1
	45-hydroxy-1a-homoYTX	0.5
STX-group toxins (STX-equivalents)	STX	1
	NeoSTX	1
	GTX1	1
	GTX2	0.4
	GTX3	0.6
	GTX4	0.7
	GTX5	0.1
	GTX6	0.1
	C2	0.1
	C4	0.1
	dc-STX = 1	1
	dc-NeoSTX	0.4
	dc GTX2	0.2
	dc GTX3	0.4
PTX-group toxins (PTX2-equivalents)	PTX1	1
	PTX2	1
	PTX3	1
	PTX4	1
	PTX6	1
	PTX11	1
DA and its isomers	None established	-

The EFSA Journal (2009) 1306, 1-23

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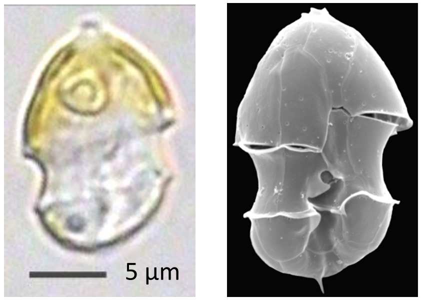
Variability of phycotoxins

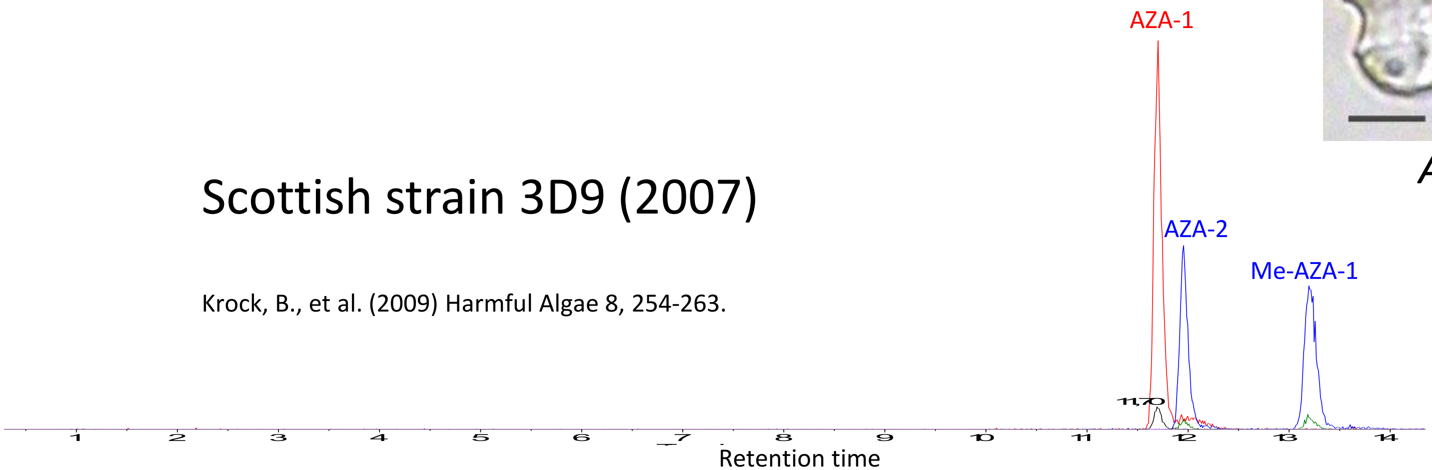
Azadinium as AZA producer



A. spinosum

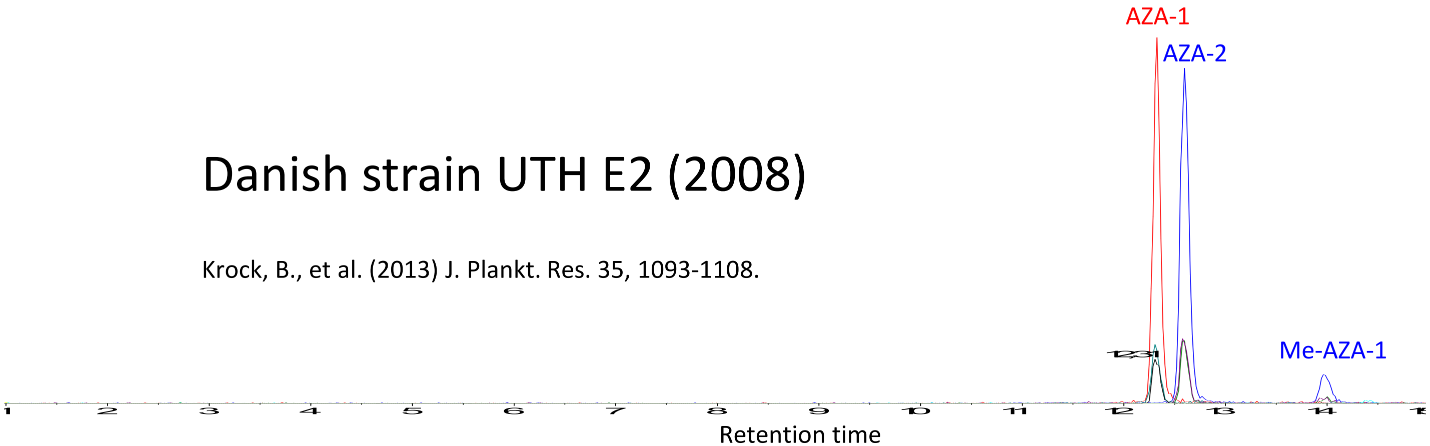
Scottish strain 3D9 (2007)

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



Danish strain UTH E2 (2008)

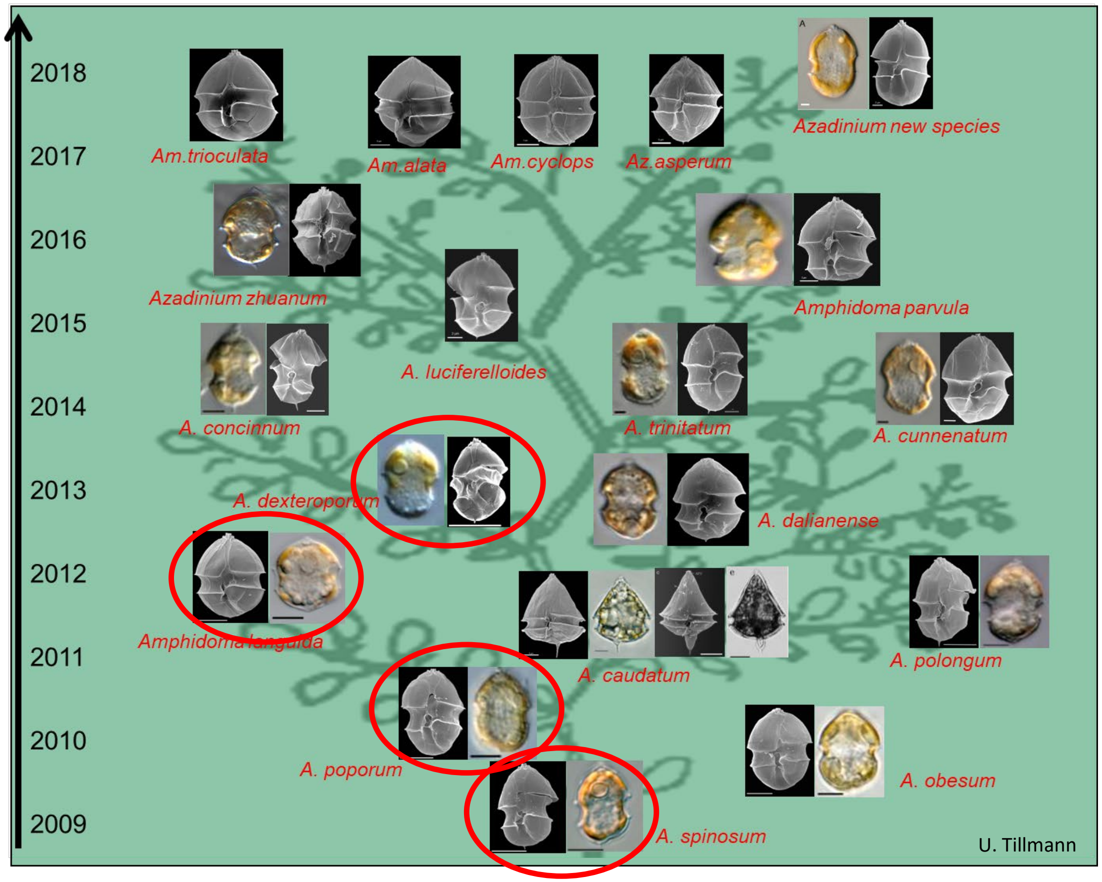
Krock, B., et al. (2013) J. Plankt. Res. 35, 1093-1108.

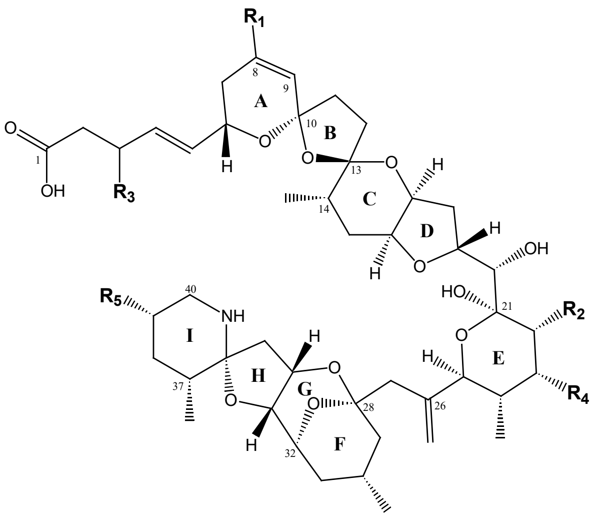


m/z 842>824

m/z 856>838

Variability of phycotoxins





Variability of phycotoxins

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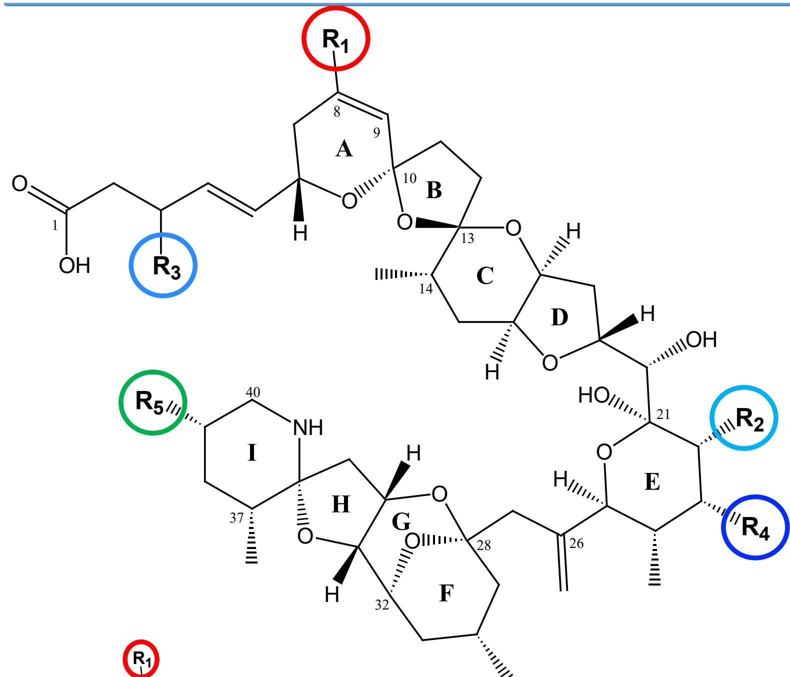
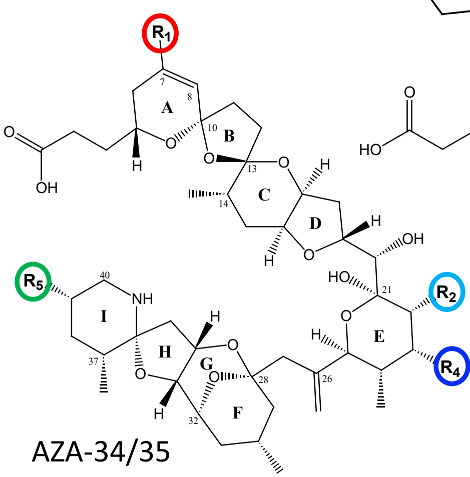
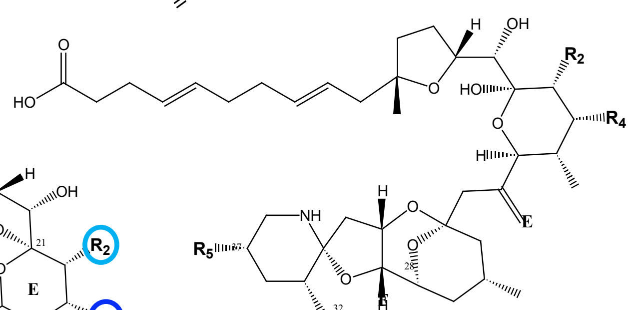




AZA-34/35



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

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Variability of phycotoxins

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Currently Known AZA from Dinoflagellate (Algal) Origin

#	AZA	*m/z* [M+H]$^+$	*m/z* group 4 fragment	*m/z* group 5 fragment	Producer	Reference
1	AZA-1	842	362	262	*A. spinosum*	Krock et al. 2009
2	AZA-2	856	362	262	*A. spinosum* *A. poporum* *Am. languida*	Krock et al. 2009 Krock et al. 2014 Tillmann et al. 2017
3	epi-AZA-7	858			*A. dexteroporum*	Rossi et al. 2017
4	AZA-11	872			*A. poporum*	Krock et al. 2014
5	AZA-33	716			*A. spinosum*	Kilcoyne et al. 2014
6	AZA-34	816	[illegible]	[illegible]	*A. spinosum*	{Kilcoyne et al. 2014
7	AZA-35	830	362	262	*A. spinosum* *A. dexteroporum*	Kilcoyne et al. 2014 Rossi et al. 2017
8	AZA-36	858	[illegible]	[illegible]	*A. poporum*	Krock et al. 2015
9	AZA-37	846			*A. poporum*	Krock et al. 2015
10	AZA-38	830			*Am. languida*	Krock et al. 2012
11	AZA-39	816			*Am. languida*	Krock et al. 2012
12	AZA-40	842			*A. poporum*	Krock et al. 2014
13	AZA-41	854	360	260	*A. poporum*	Krock et al. 2014
14	AZA-42	870	360	260	*A. poporum*	Krock et al. under review
15	AZA-43	828	[illegible]	[illegible]	*Am. languida*	Tillmann et al. 2017
16	AZA-50	842			*A. spinosum*	Tillmann et al. 2018
17	AZA-51	858			*A. spinosum*	Tillmann et al. 2018
18	AZA-52	830			*Am. languida*	Tillmann et al. 2018
19	AZA-53	830			*Am. languida*	Tillmann et al. 2018
20	AZA-54	870			*A. dexteroporum*	Rossi et al. 2017
21	AZA-55	868			*A. dexteroporum*	Rossi et al. 2017
22	AZA-56	884	362	262	*A. dexteroporum*	Rossi et al. 2017
23	AZA-57	844	362	262	*A. dexteroporum*	Rossi et al. 2017
24	AZA-58	828	362	262	*A. dexteroporum*	Rossi et al. 2017
25	AZA-59	860	362	262	*A. poporum*	Kim et al. 2017
26	AZA-62	870	362	262	*A. poporum*	Krock et al. 2019

26 AZAs from planktonic origin

Currently
62 published AZAs

And at least additional
10 known AZAs

Variability of phycotoxins

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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

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

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!

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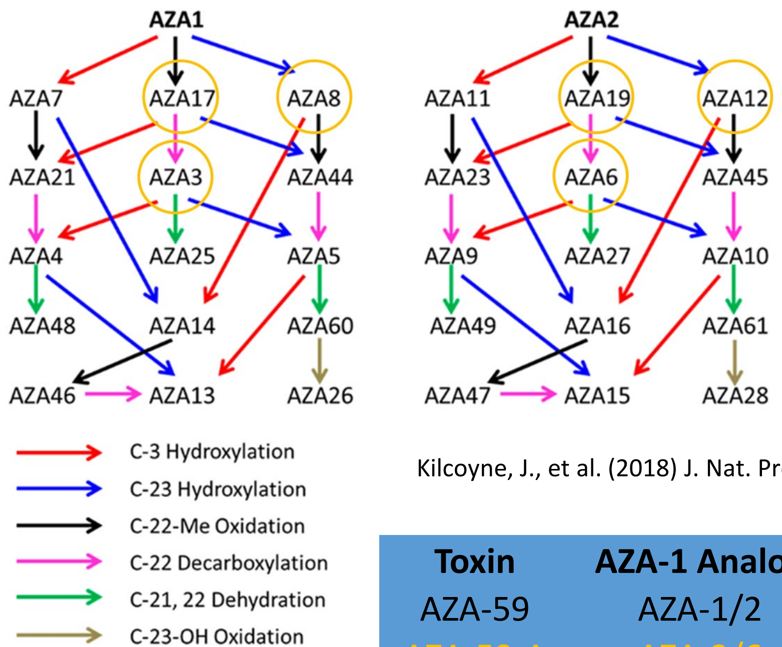
Variability of phycotoxins

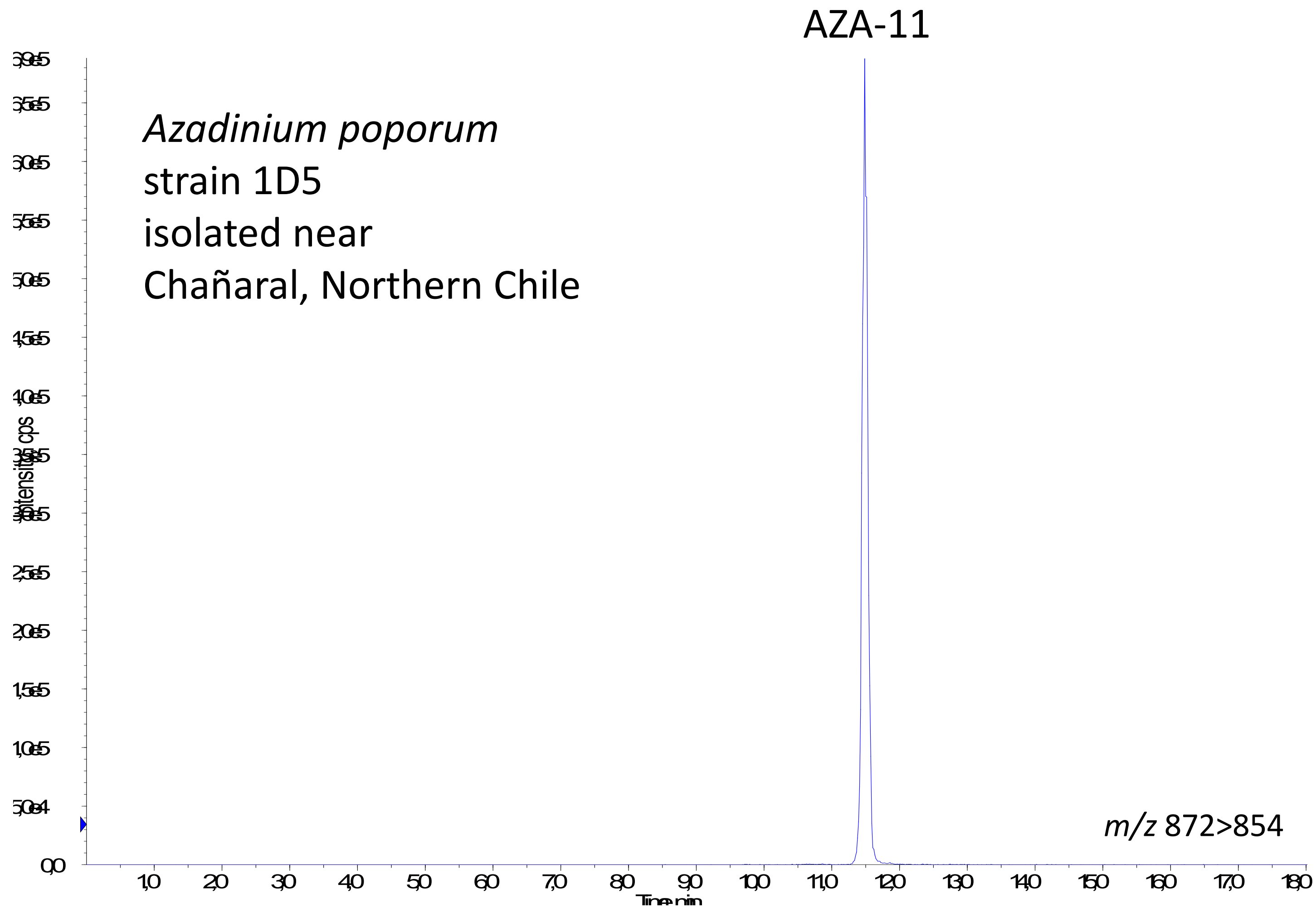
Azaspiracids – Metabolism in Bivalves



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	10,6
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

Variability of phycotoxins



Tillmann et al. (2017) J. Plankt. Res 39(2): 350-367)

Variability of phycotoxins

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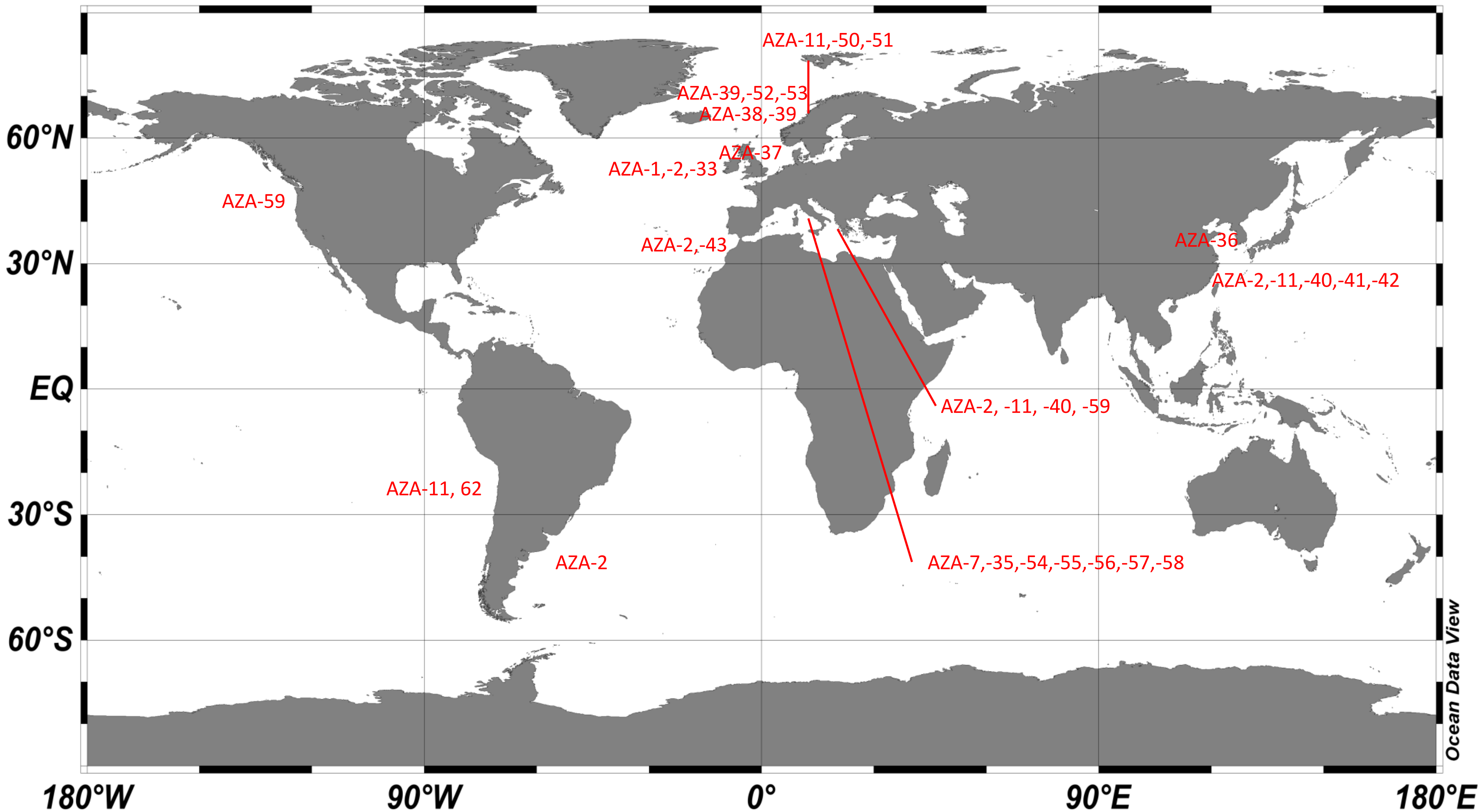
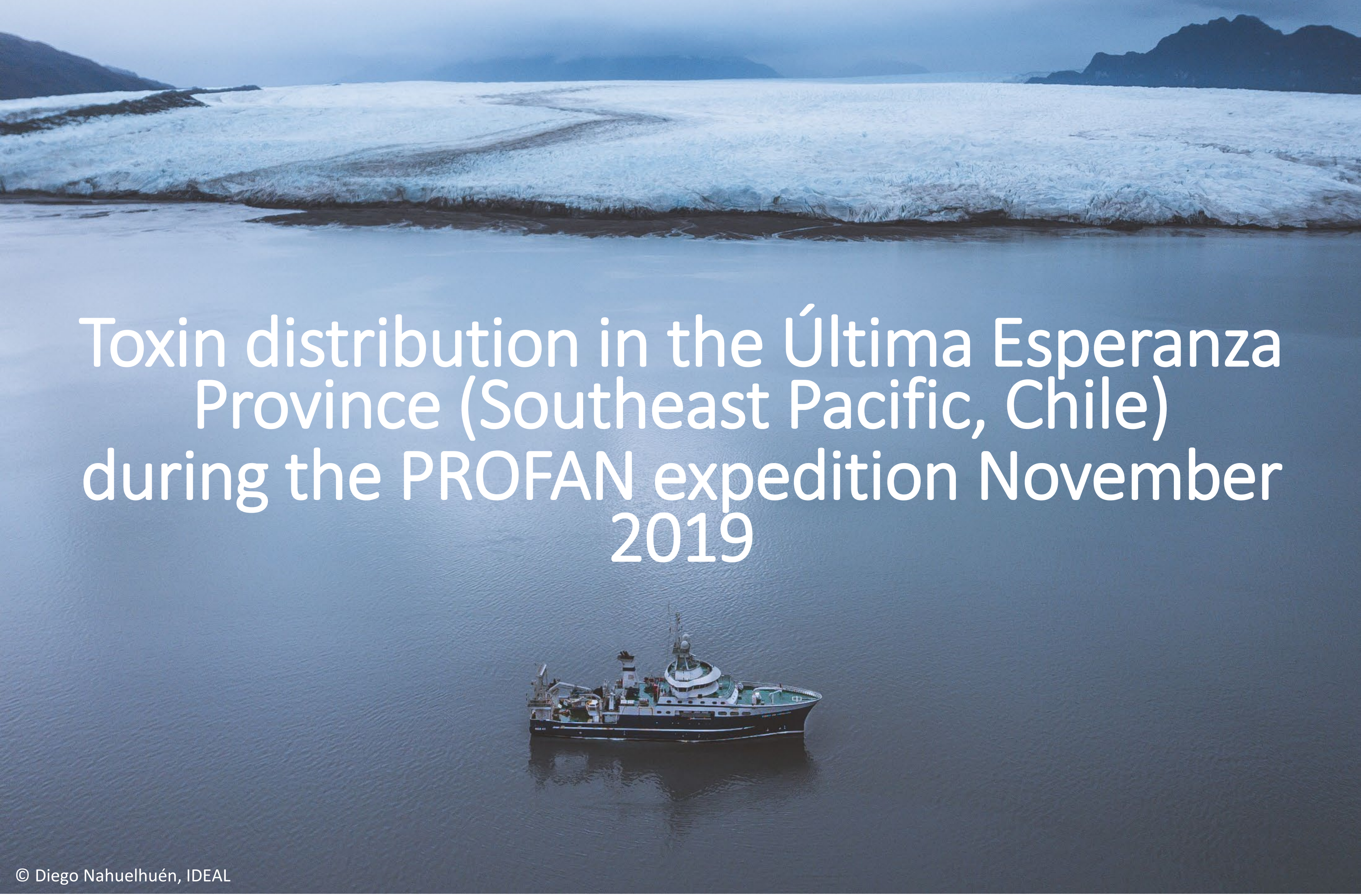
Azaspiracids – Geographic distribution



Toxin distribution in the Última Esperanza Province (Southeast Pacific, Chile) during the PROFAN expedition November 2019



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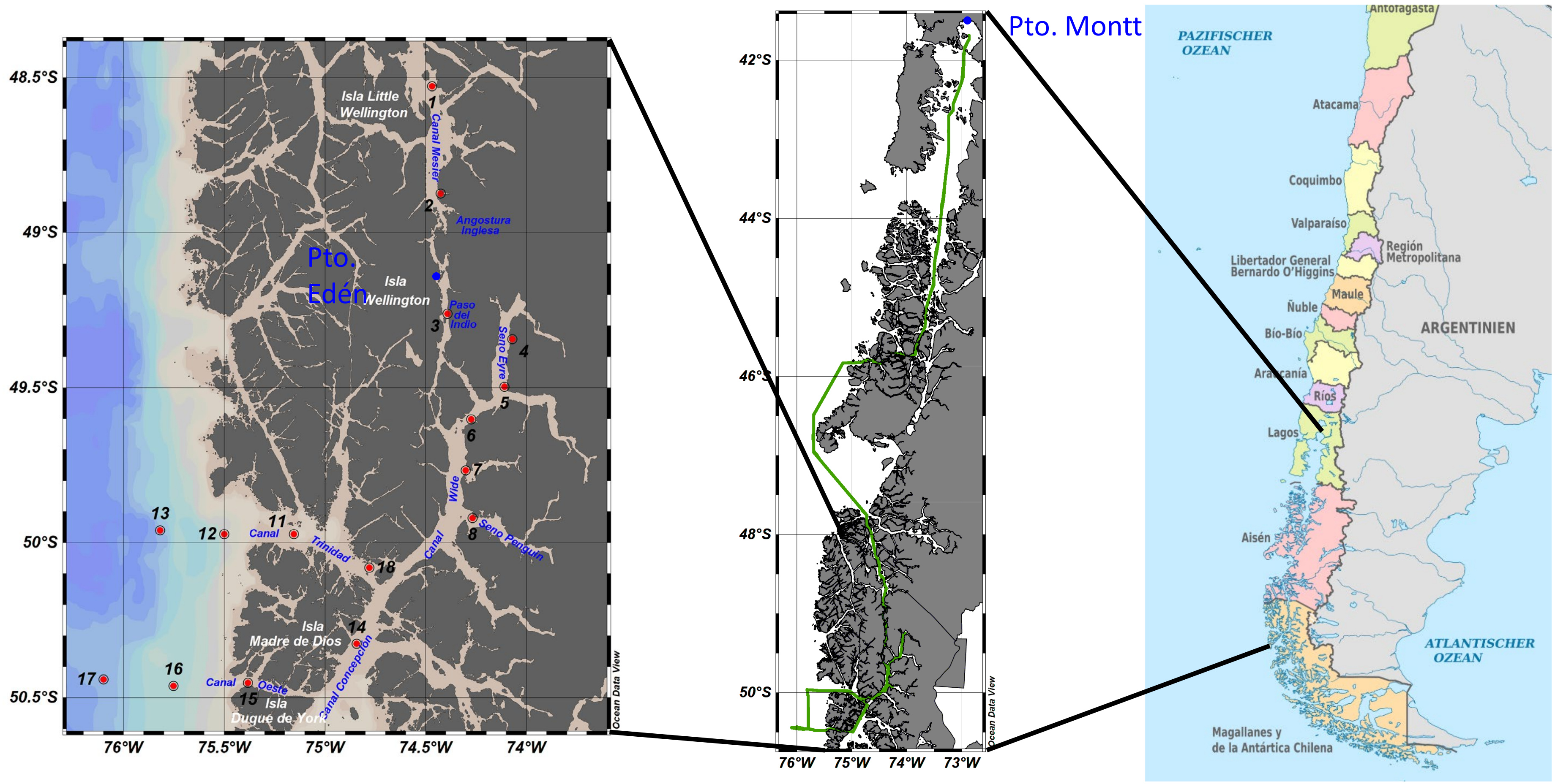
Study area



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PROFAN expedition (November 2019)

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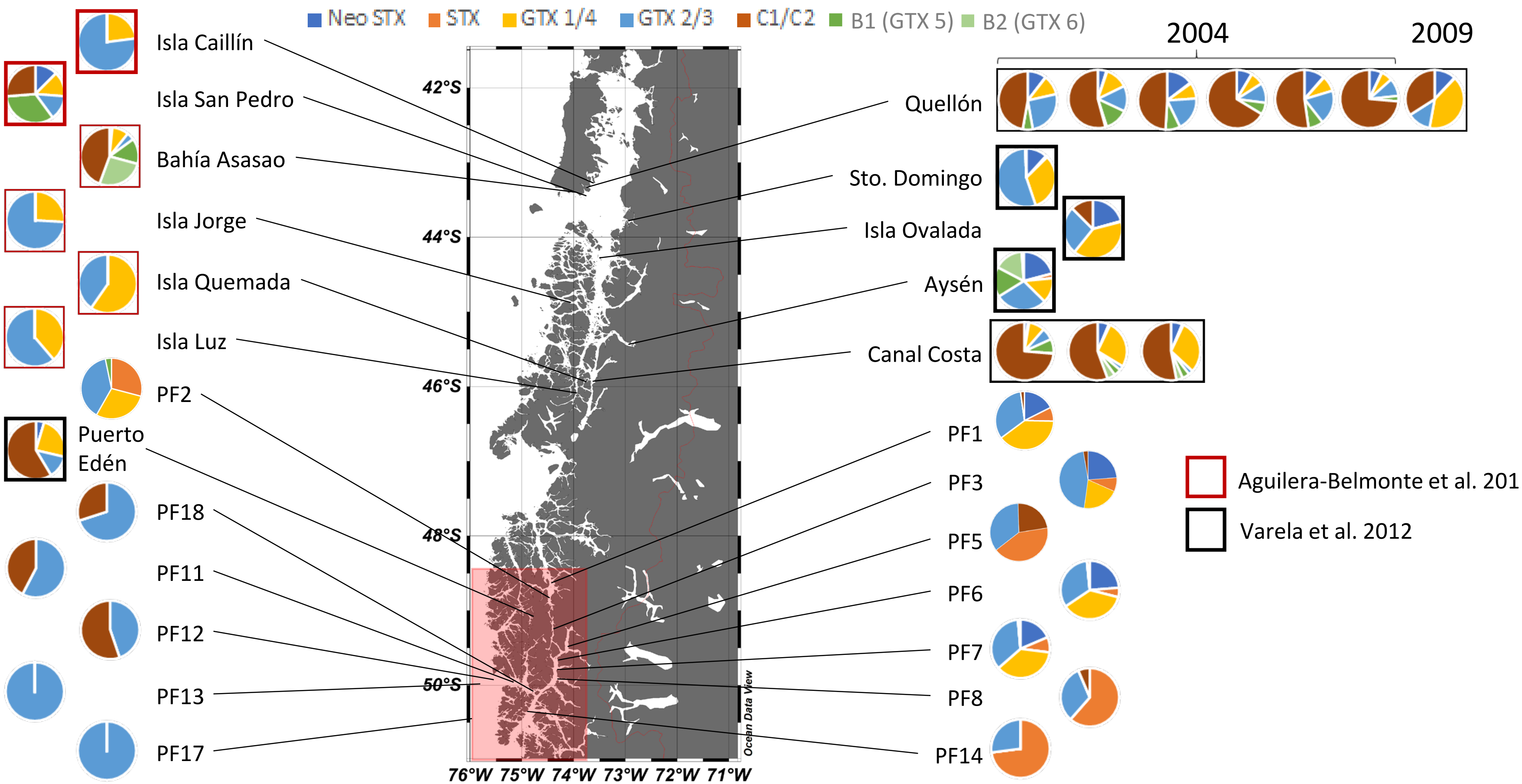
1. PSP-toxin – relative profiles



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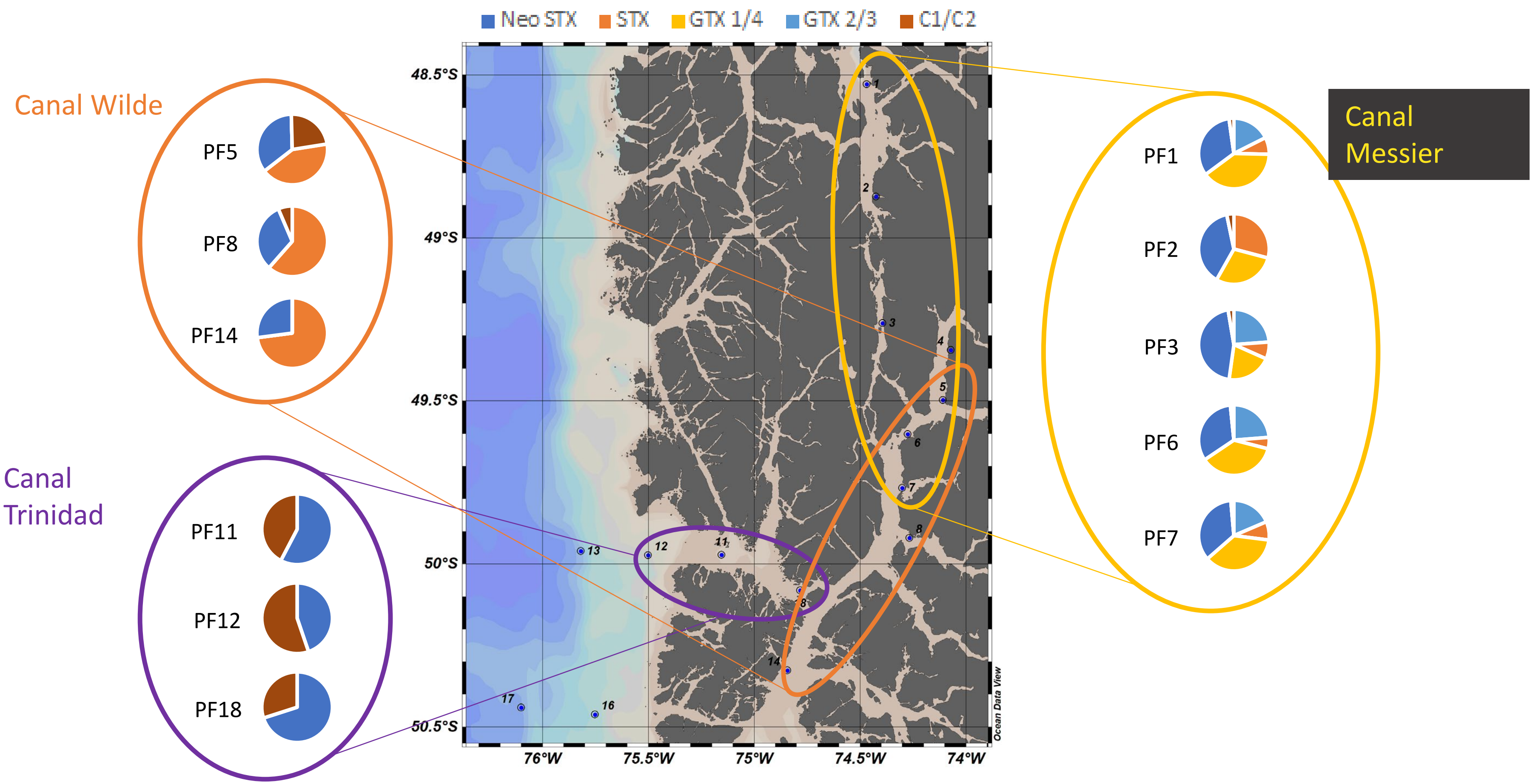
1. PSP-toxins – geographic distribution of profiles



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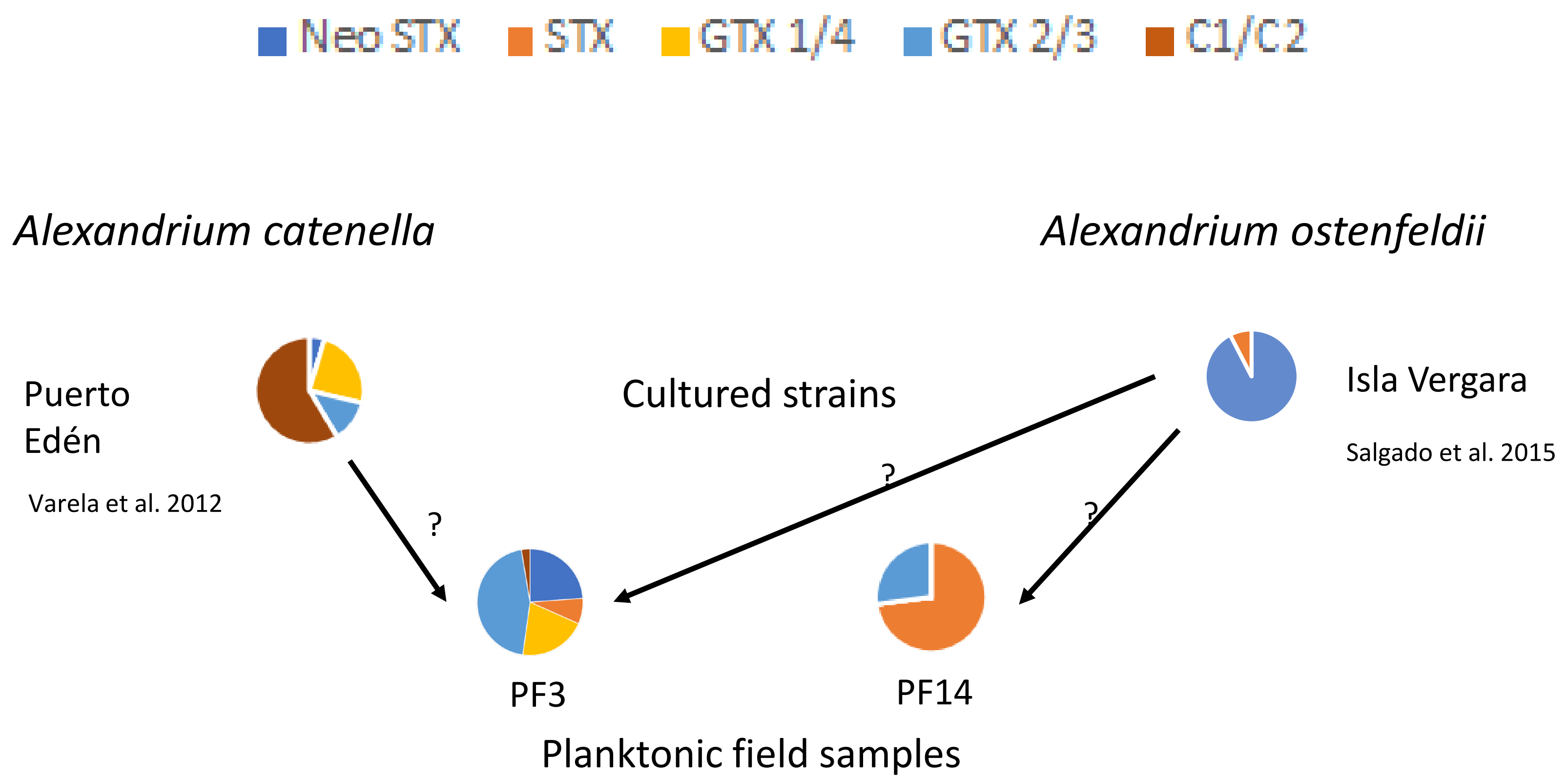
1. PSP-toxins – species contributions?



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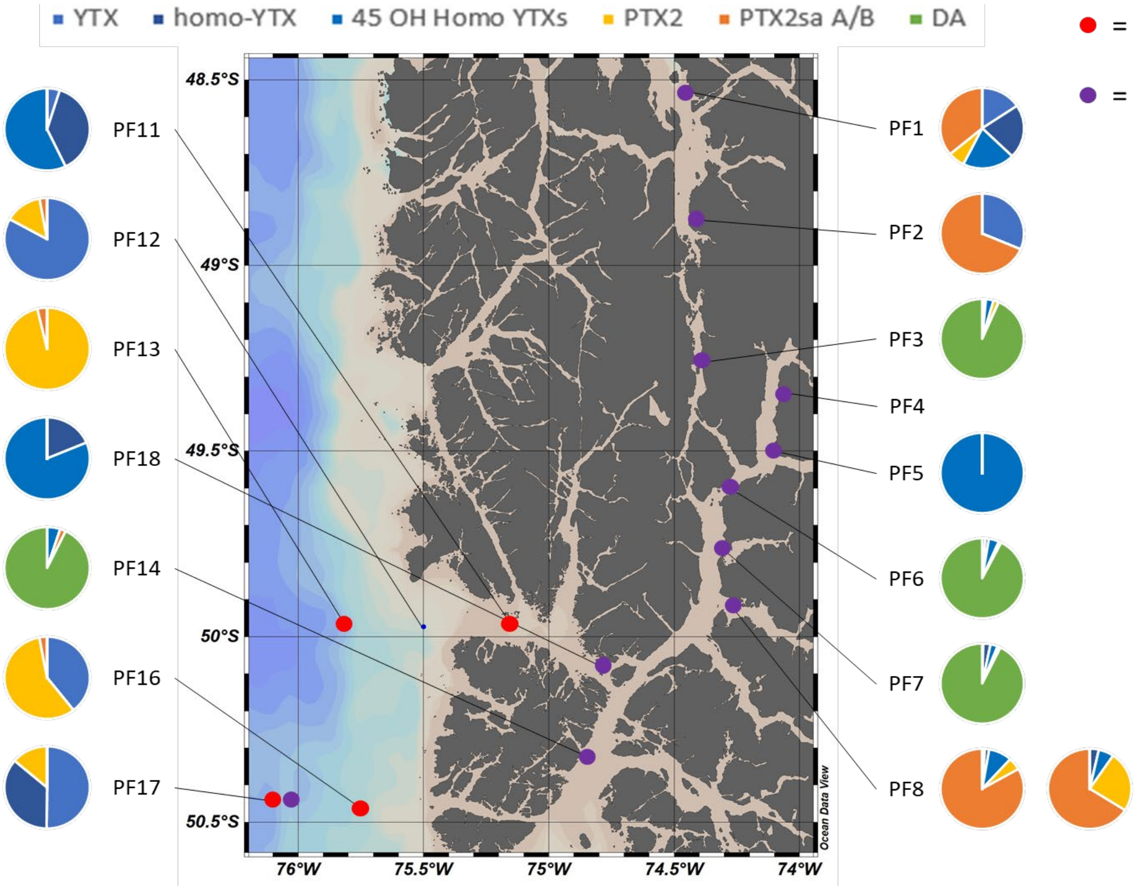
2. Lipophilic toxins – qualitative profiles



DA only at inner stations

Coincidence of YTX, homo-YTX and OH-homo-YTX

Presence of PTX-2sa in plankton

Presence of 20-Me SPX G almost exclusively at inner stations; first report of 20-Me SPX G in Chile

AZA-2 only at outer stations

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3. SPATT sampling

SPATT = Solid Phase Adsorption Toxin Tracking

- Water is pumped through a porous synthetic resin
  → passively adsorbing toxins

- 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 iformation on the current plankton presence

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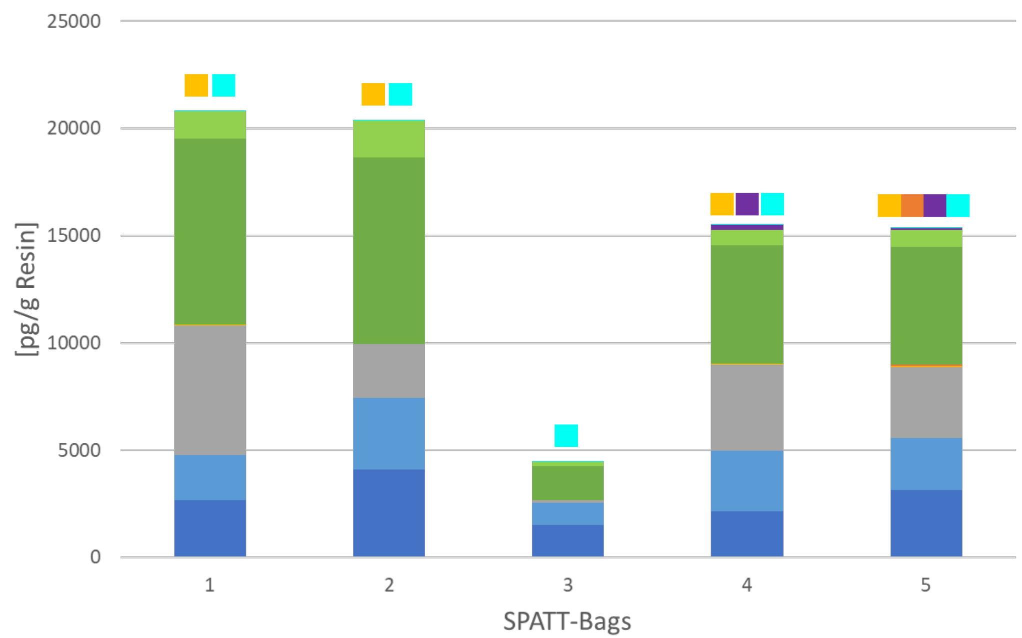
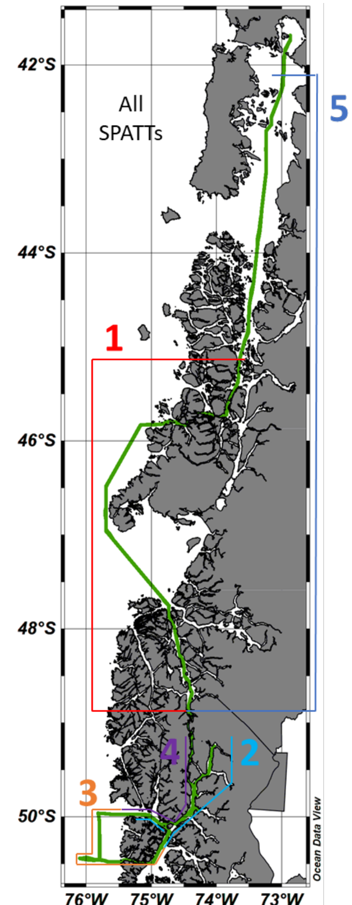
3. SPATT sampling



Möller et al. (2022) Prog. Oceanogr., in revision



Toxin abundance higher in channels and fjords than in the open Pacific

OA/DTX-1 were detetced during the entire track and were not restricted to Aysén Region

AZA-2 was present during the entire track at low abundances

First report of pinnatoxin G in Chile

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PROFAN expedition (November 2019)

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Main results

1. Phycotoxins were detected at all stations except for station 4 (Eyre Gulf, Pio XI Glaciar)
2. Greographically discinct distribution of PSP toxin profiles in Úlima Esperanza Province
3. DA, PTXs and YTXs were most abundant lipophilic toxins
4. Presence of homo-YTXs indicates the presence of other YTX-producing species than *Protoceratium reticulatum*
5. Even though no OA/DTX-1 were detected in plankton samples, these toxins were present in water samples during the entire cruise transect and indicate the presence of *Dinophysis acuta* also in the Magallanes Region
6. AZA-2 was detected in low concentrations in water samples during the entire cruise transect and seems to be the prevalent AZA variant in southern Chile
7. Pinnatoxin G (PnTx G) was detected for the first time in Chilean waters and stongly indicates the presence of *Vulcanodinium rugosum*

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M179
FjordFlux

Further information:
https://m.dw.com/es/una-ventana-al-futuro-desde-la-patagonia-la-campaña-trilateral-fjord-flux/a-60584473

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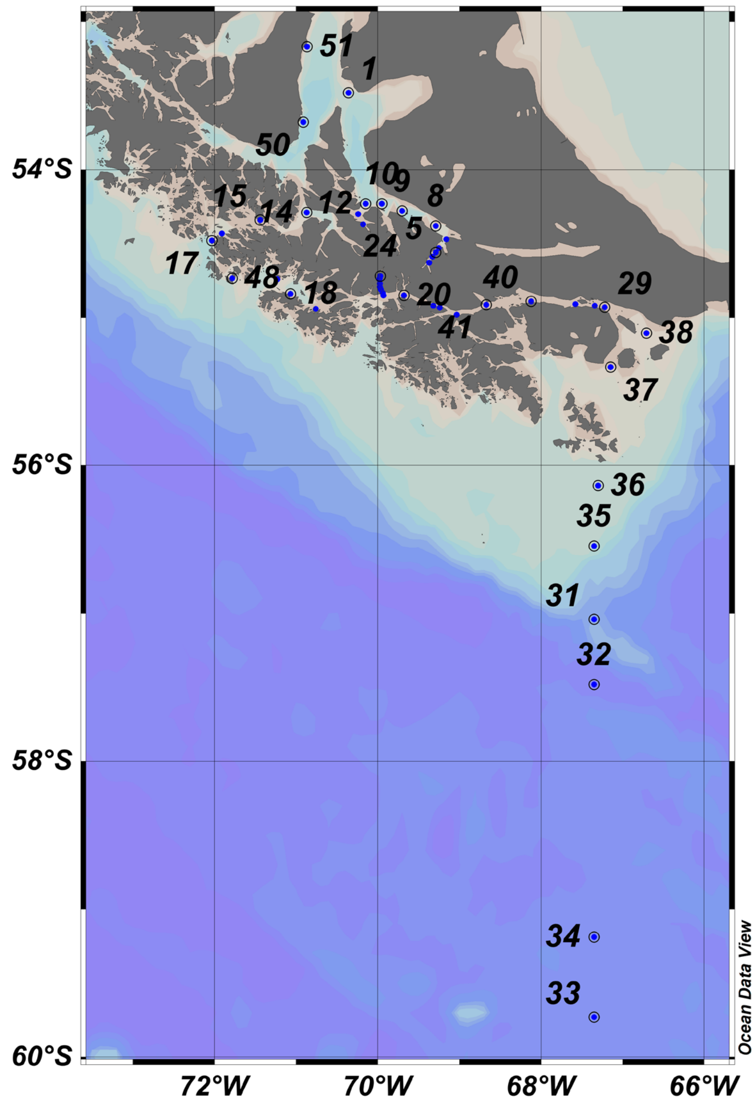

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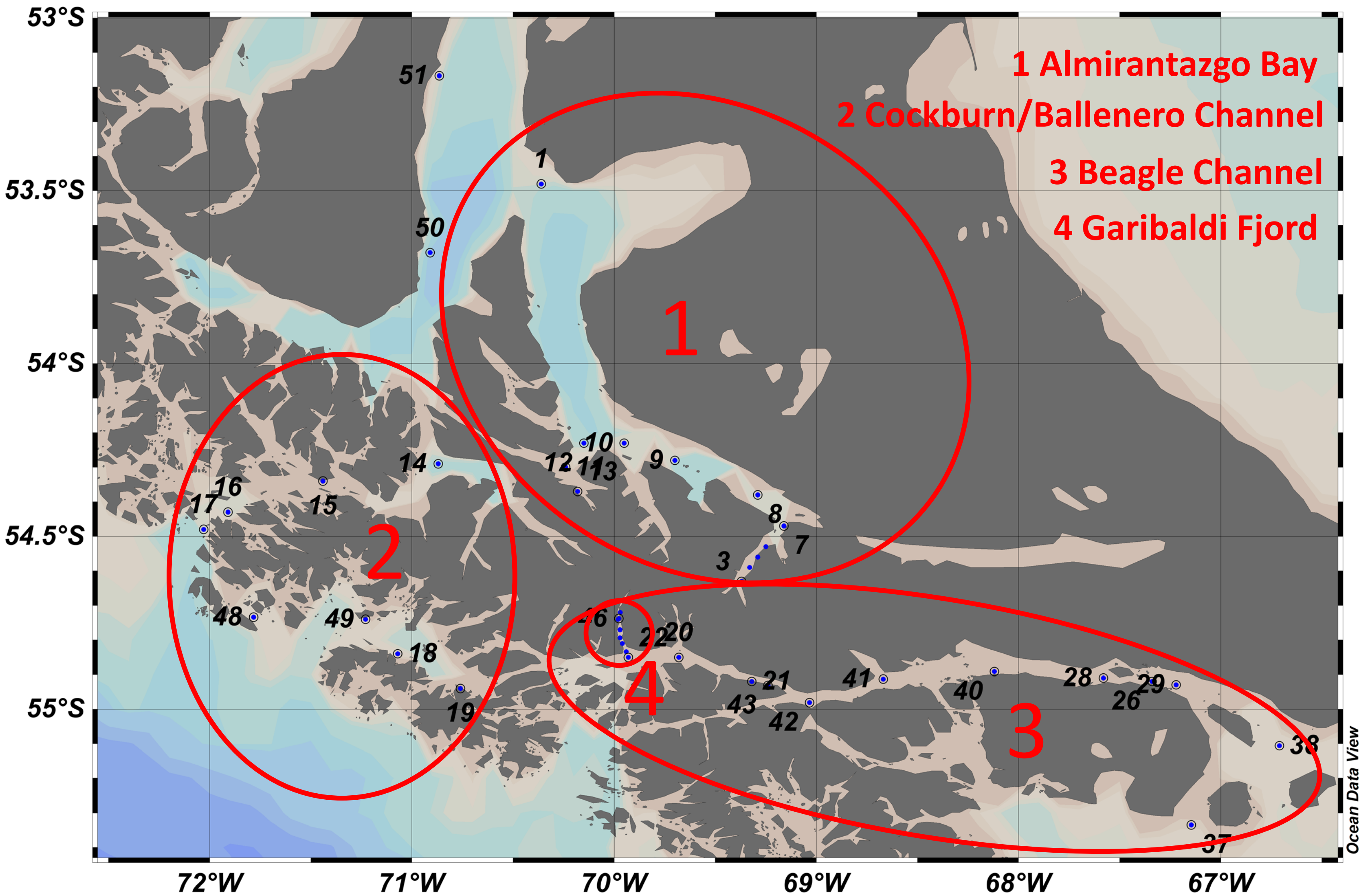

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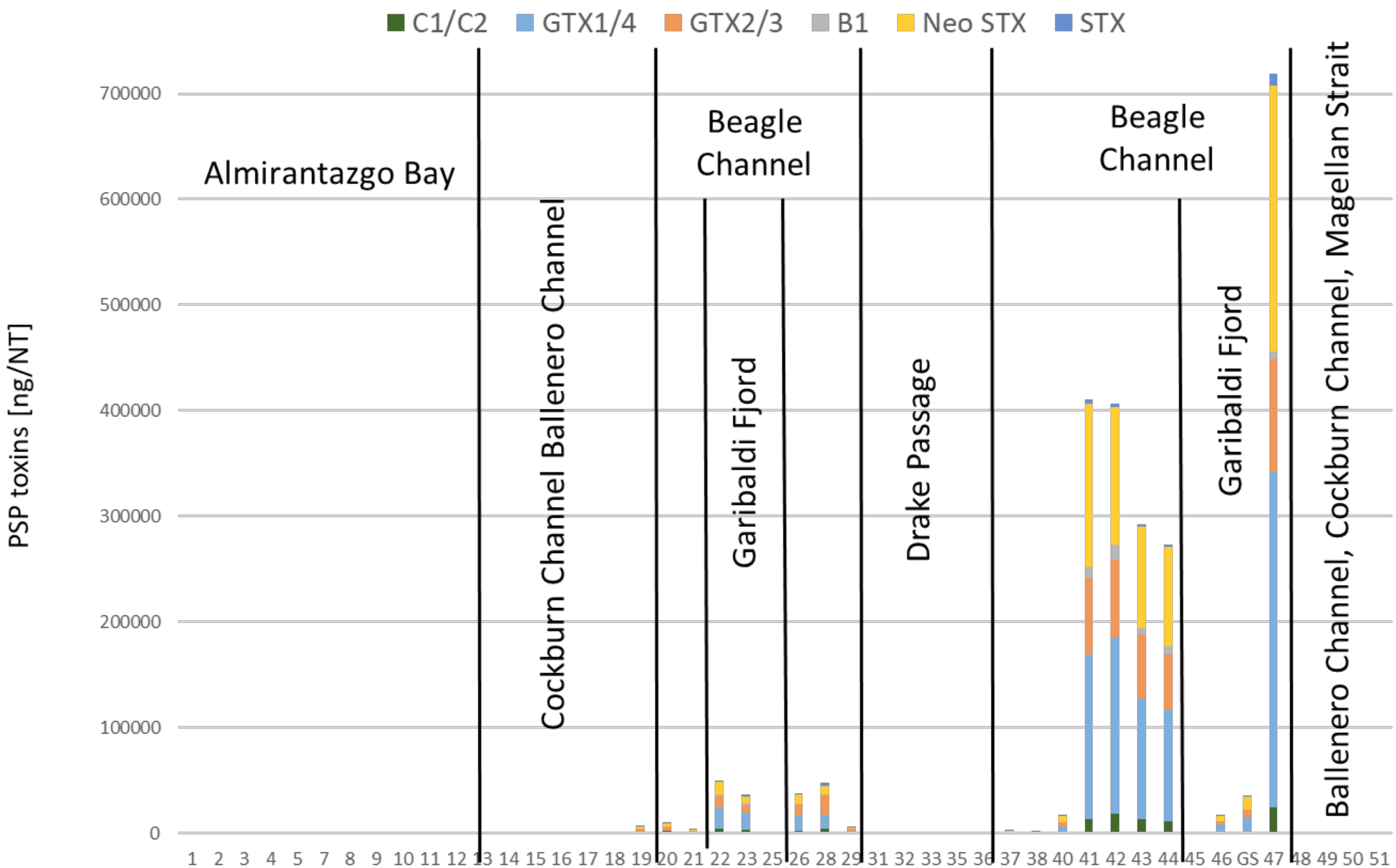

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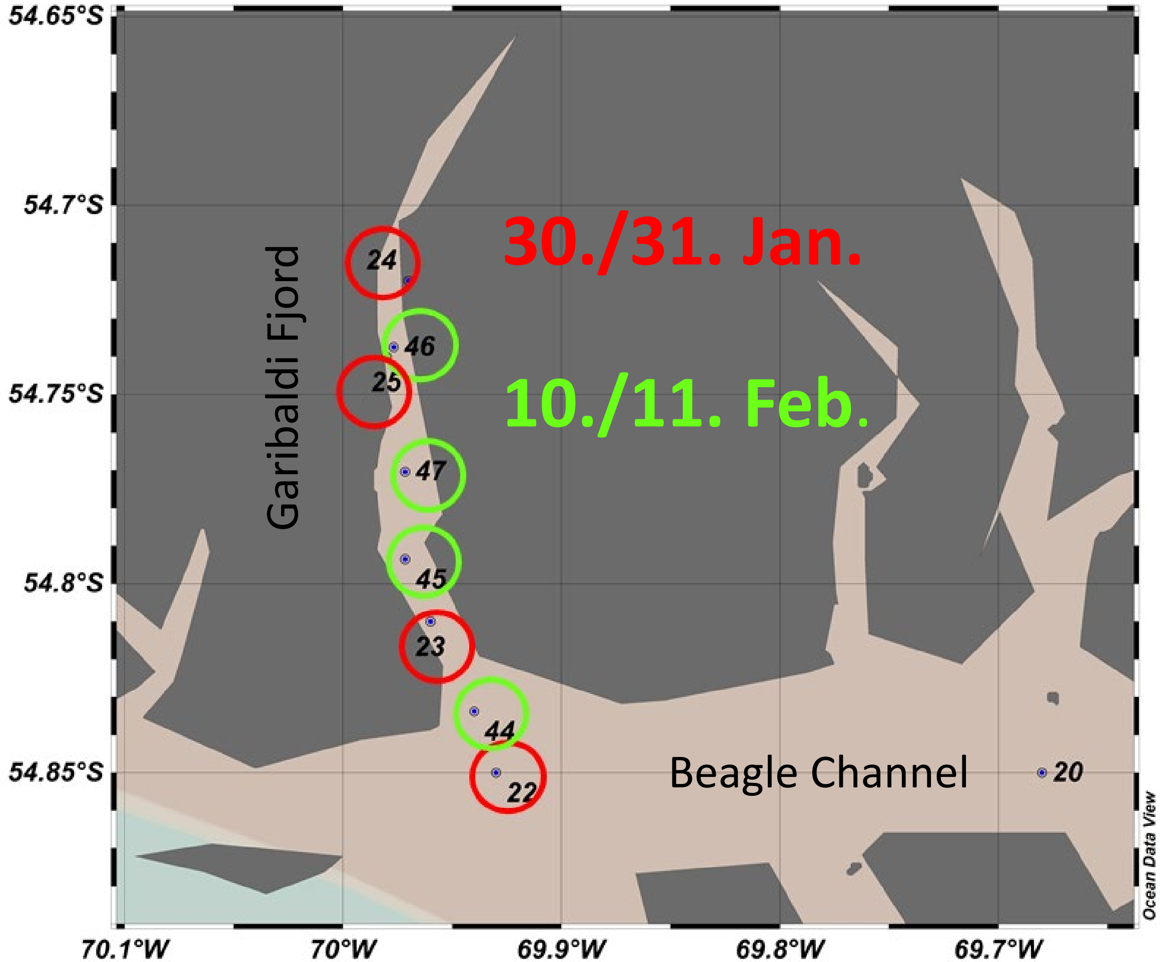

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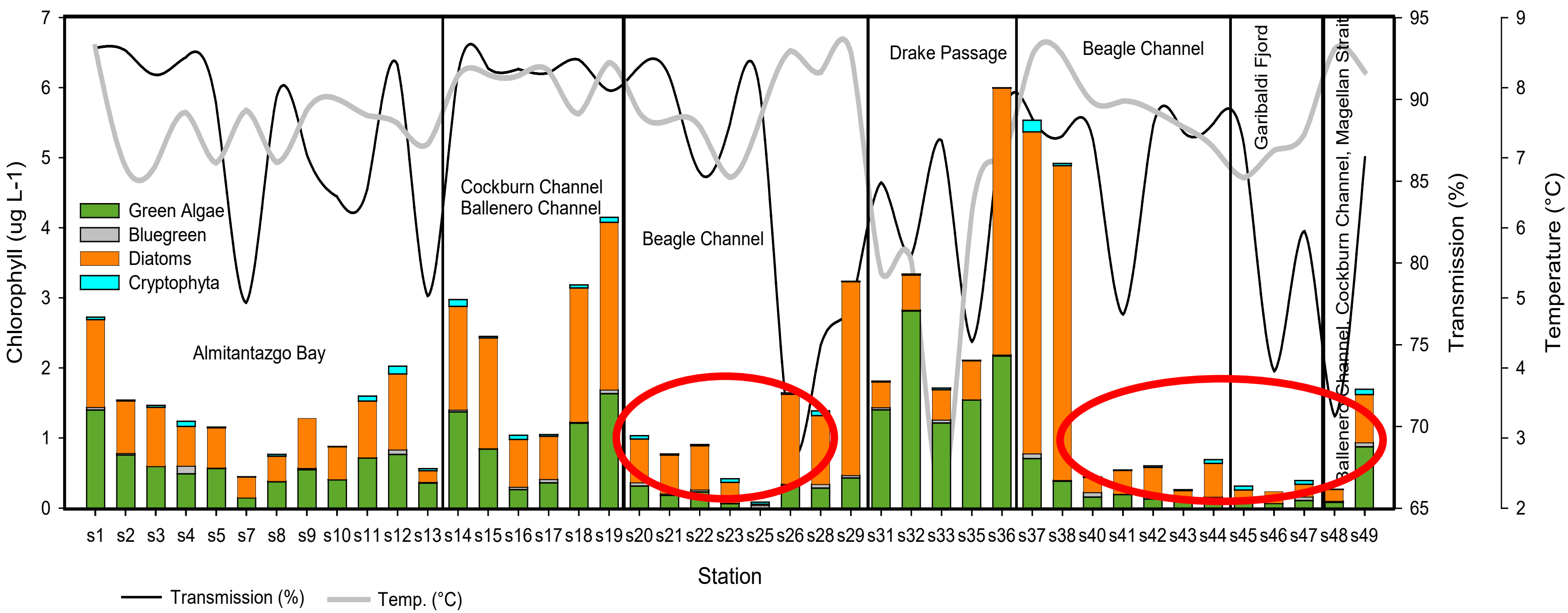
Functional plankton groups



Data: Ricardo Giesecke, IDEAL

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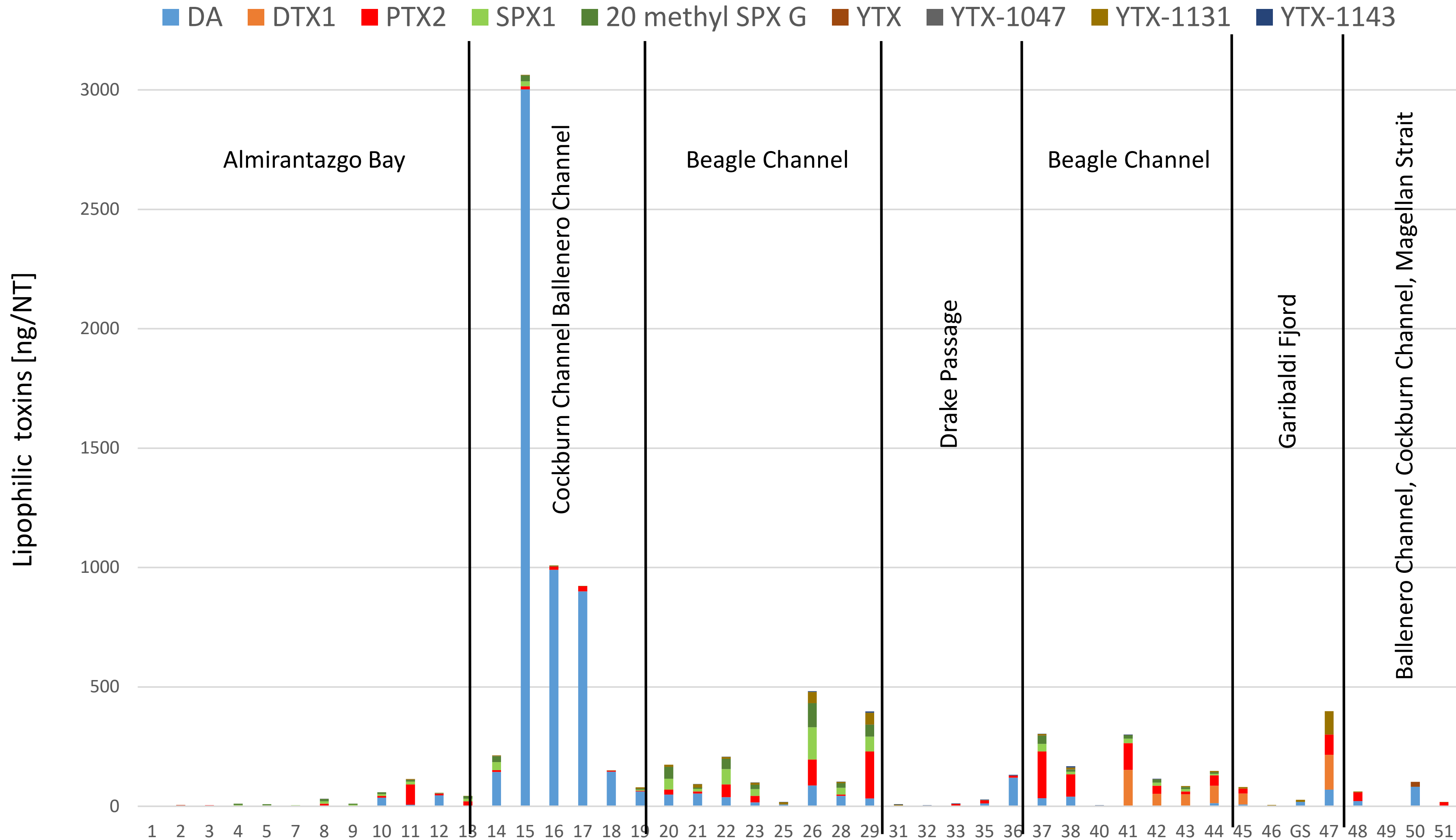

XLI Congreso Chileno de Ciencias del Mar, UCSC, Concepción, 23-27 Mayo 2022, B. Krock

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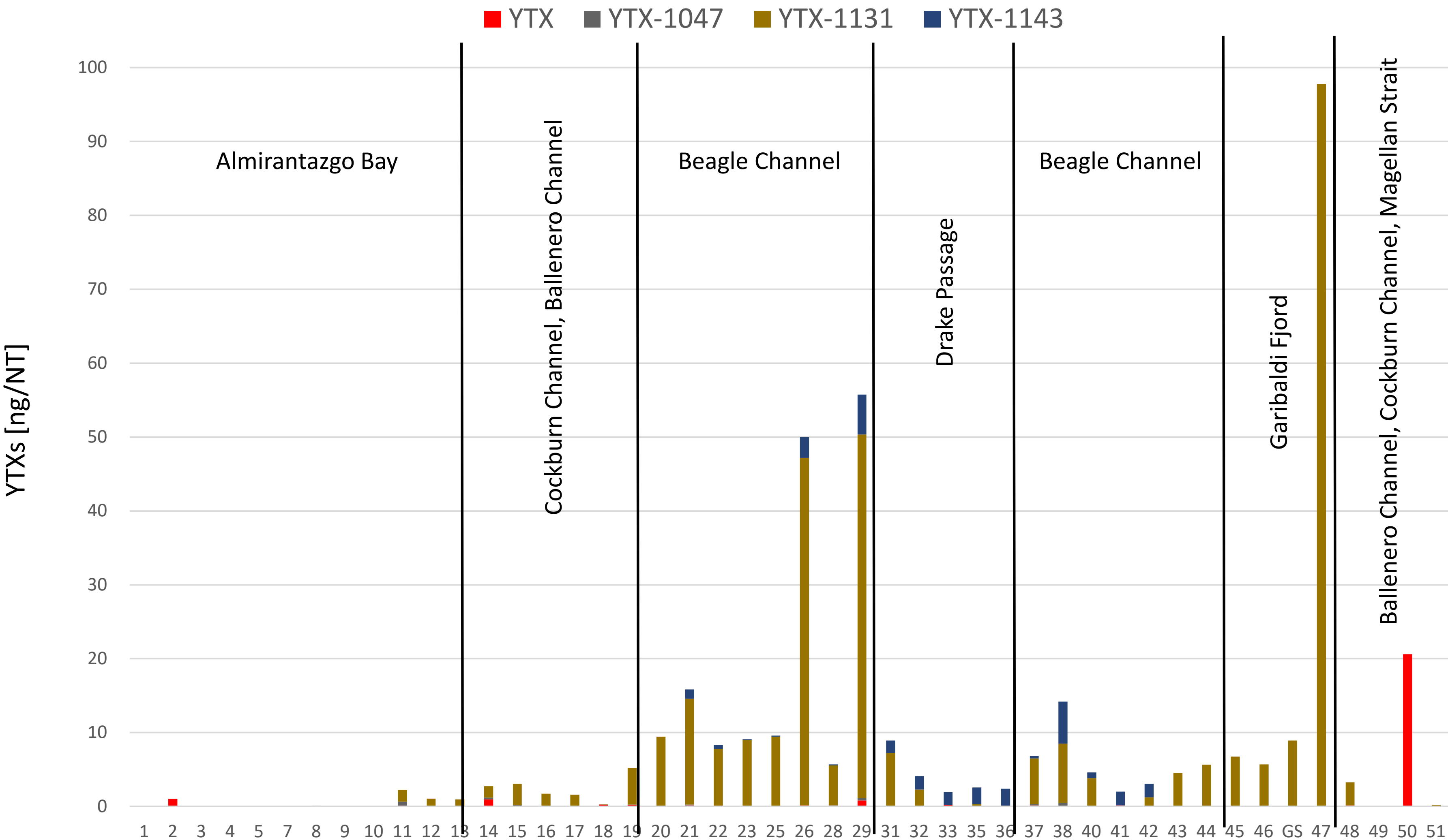

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Conclusions

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1. Ichthyotoxins are rarely characterized, but pose an increasing threat to increasing aquacultural activities

2. Phycotoxin variability of known toxin classes is high and yet not fully explored, especially in hard to access coastal areas in Southern Chile

3. Phycotoxin profiles tend to be geographically relatively stable

4. Locally occurring phycotoxin variants should be included in chemical monitoring efforts in addition to officially regulated toxins

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Any
Questions?
Thanks for
Your Attention!