



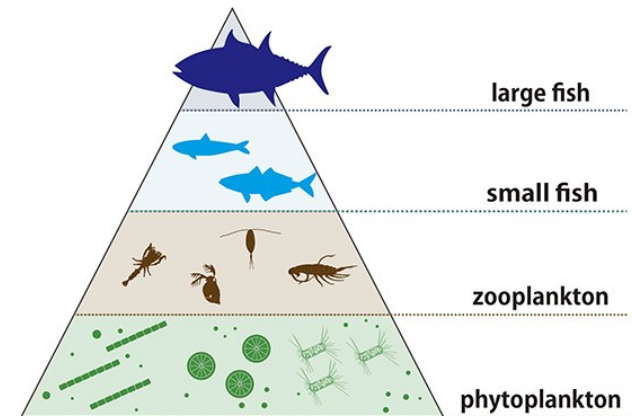
# What do we know about ichthyotoxic microalgal species and their toxins?

# Introduction

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



Algal Bloom Lake Erie



# Introduction

February/March 2016, Región de Los Lagos: massive bloom of *Pseudochattonella* cf. *verruculosa* associated with fish kills





# Introduction

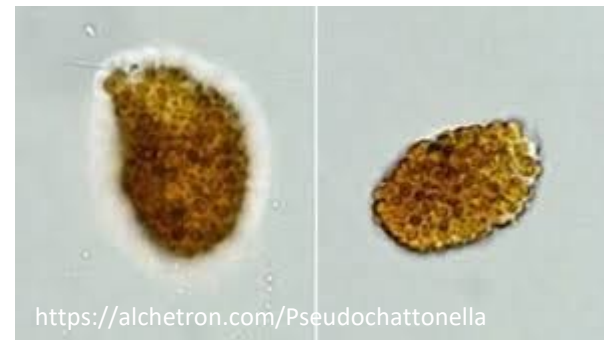
April/May 2016, Región de Los Lagos: massive bloom event of *Alexandrium catenella* associated with high shellfish contamination



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

Mechanistically both blooms were different:

*Pseudochattonella* cf. *verriculosa*:  
Ichthyotoxic



*Alexandrium catenella*:  
Paralytic Shellfish Poisoning Toxin producer



## Massive Fish Kill in the Oder River in August 2022



produced by the haptophyte *Prymnesium parvum*

## Variability

*Amphidinium* spp.: Amphidinols - 20+ known variants

*Karlodinium* spp.: Karlotoxins - 20+ known variants

*Prymnesium parvum*: Prymnesins - 100+ variants

## Other ichthyotoxic species:

*Alexandrium* spp.

*Chattonella* spp.

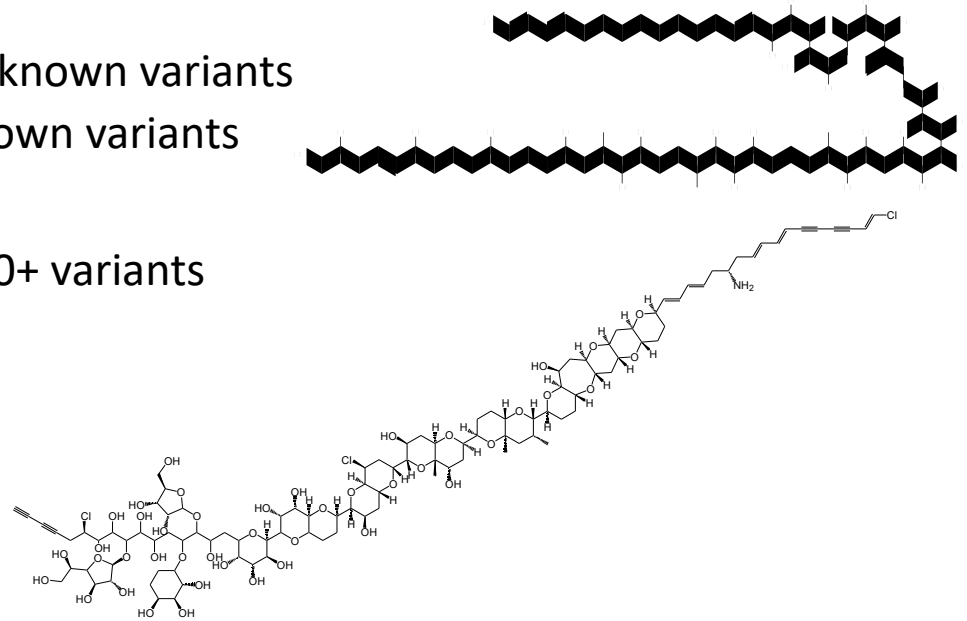
*Chrysocromulina* spp.

*Fibrocapsa japonica*

*Heterosigma akashiwo*

*Protoceratium reticulatum*

*Pseudochattonella* cf. *verruculosa*

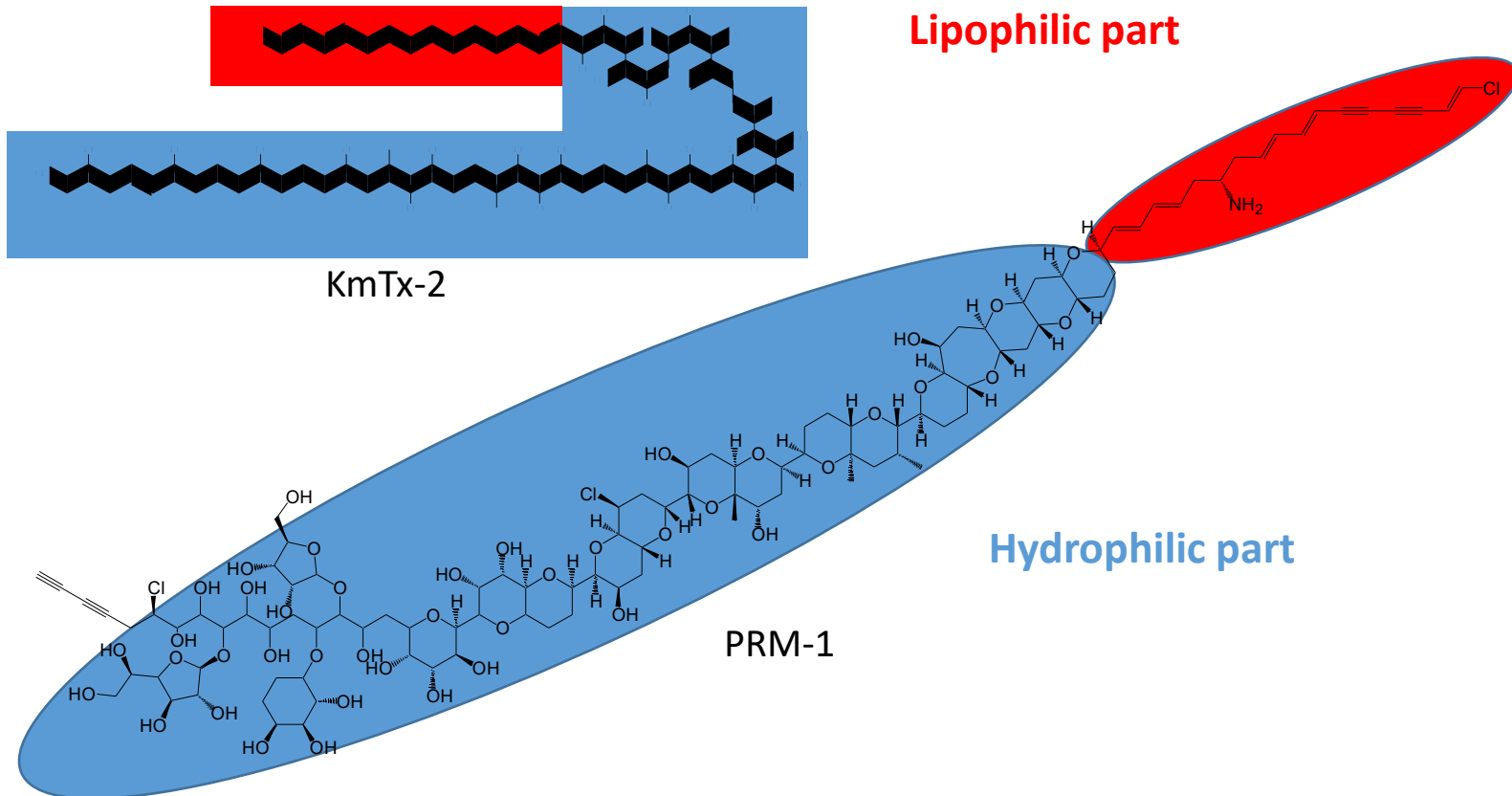


# Ichthyotoxins:

# Unknown !!

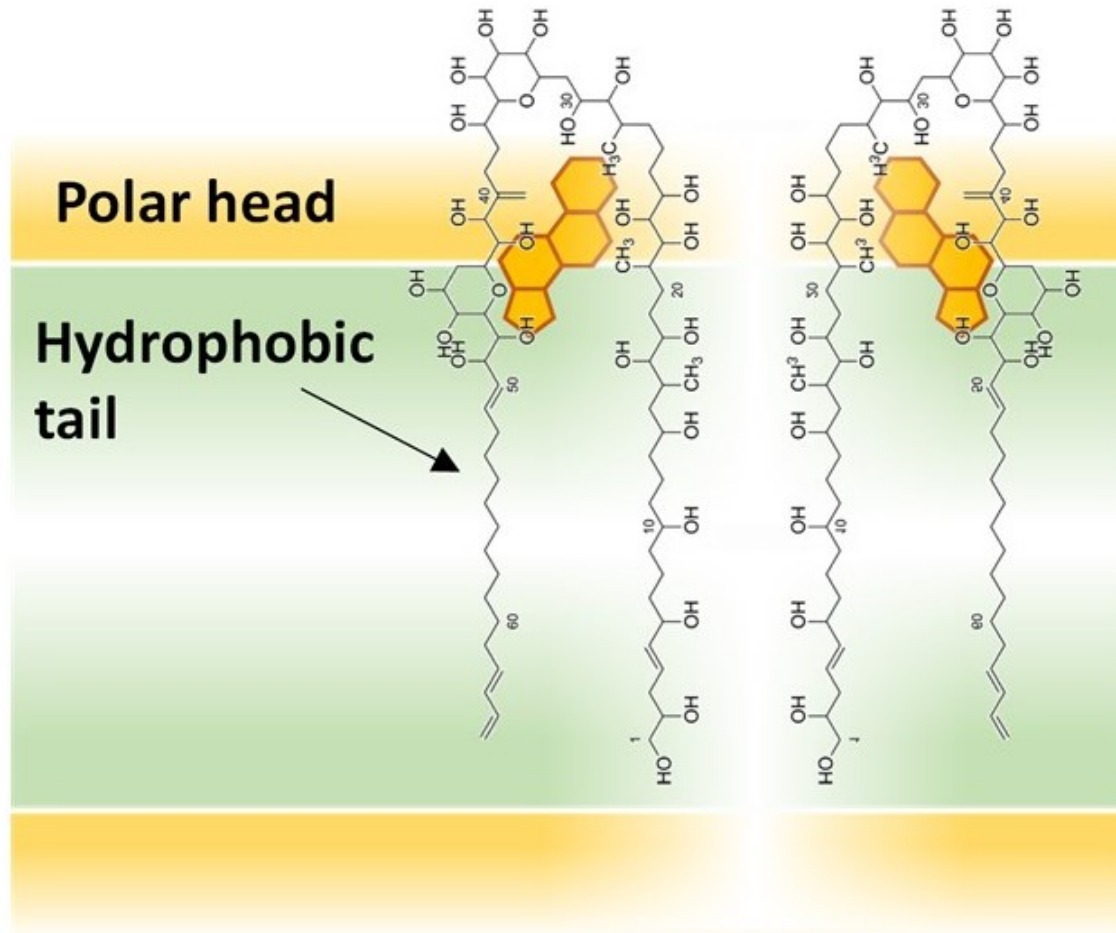
# Ichthyotoxins

## Known Ichthyotoxins





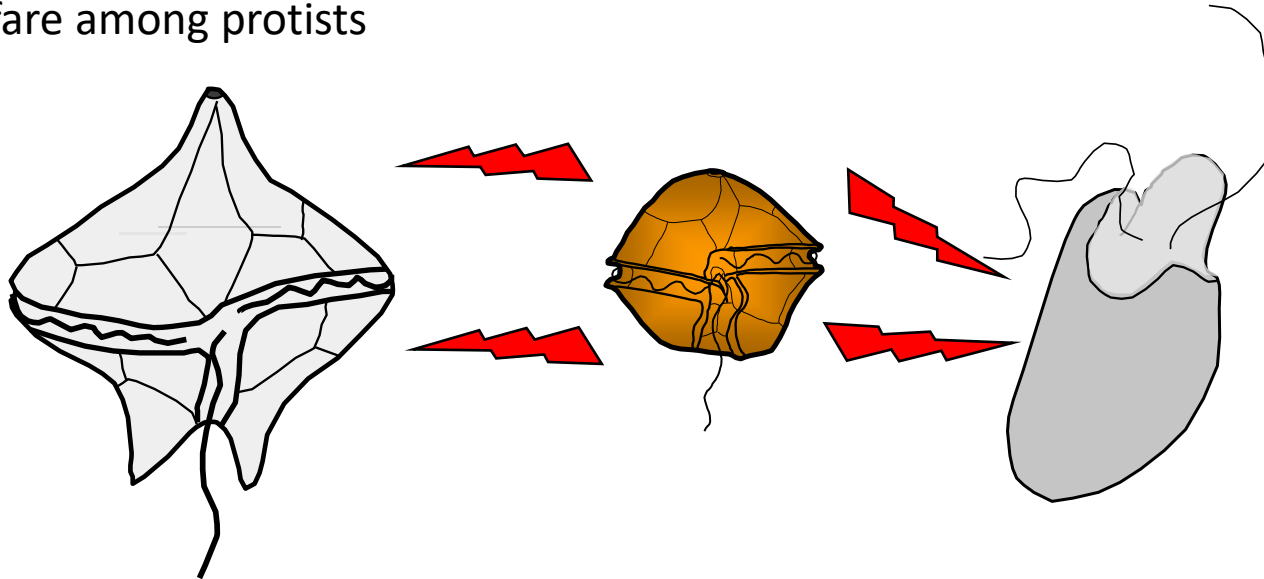
## Mode of action



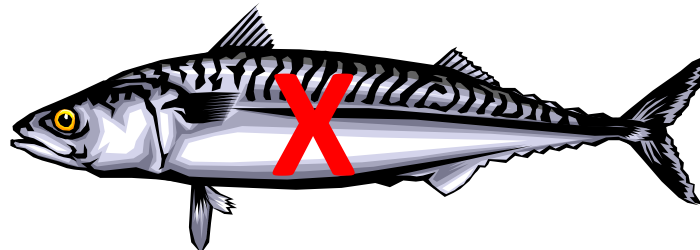
Long, M., et al. (2021) *Toxins* 13(12): 905.

## Current Hypothesis:

Bioactive extracellular compounds (BECs) of marine protists are weapons of chemical warfare among protists



Ichthyotoxicity is a collateral damage of protistan allelochemistry



# *Alexandrium catenella*

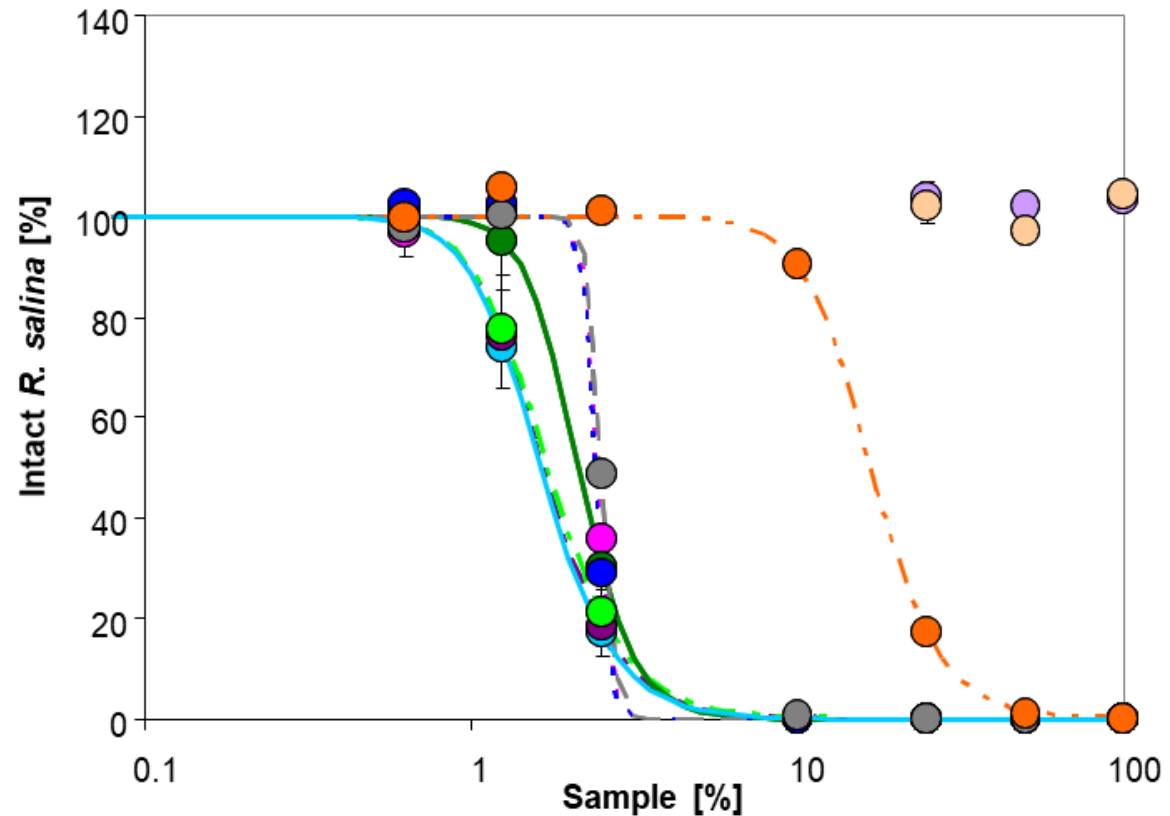
April/May 2016, Región de Los Lagos: massive bloom event of *Alexandrium catenella* associated with high shellfish contamination



## Bioactive extracellular compounds (BEC)

### Filtration

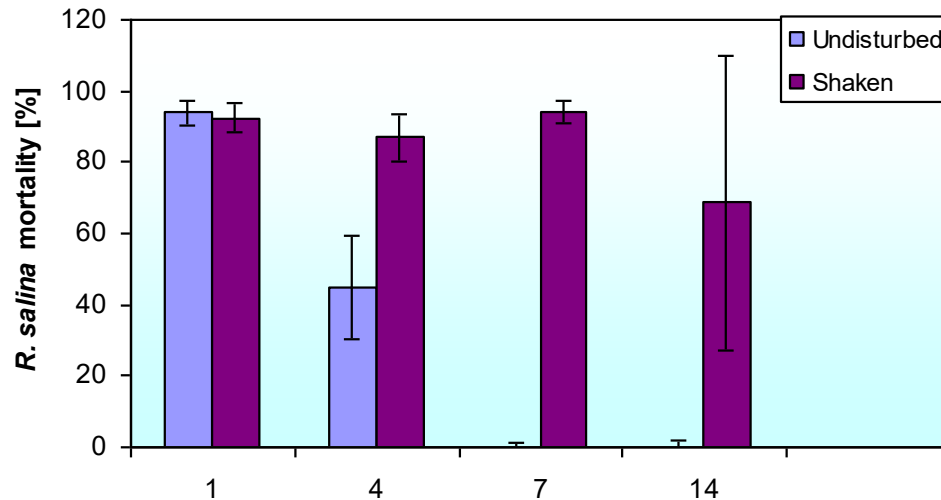
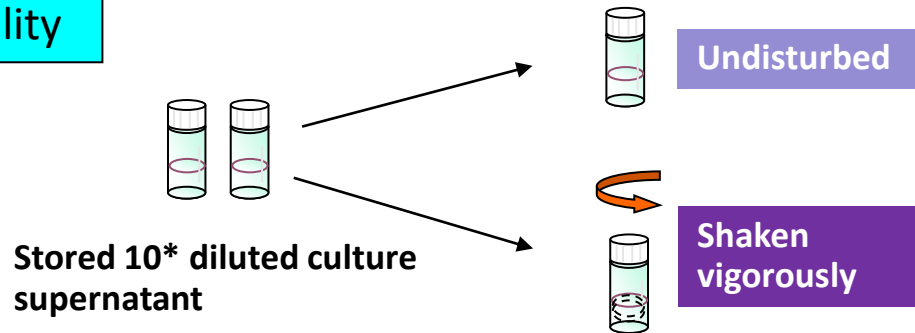
- Cell culture
- Supernatant
- 8  $\mu\text{m}$
- 5  $\mu\text{m}$
- 3  $\mu\text{m}$
- 1.2  $\mu\text{m}$
- 0.4  $\mu\text{m}$
- 0.2  $\mu\text{m}$
- 0.1  $\mu\text{m}$
- GF/C





## Bioactive extracellular compounds (BEC)

### Stability



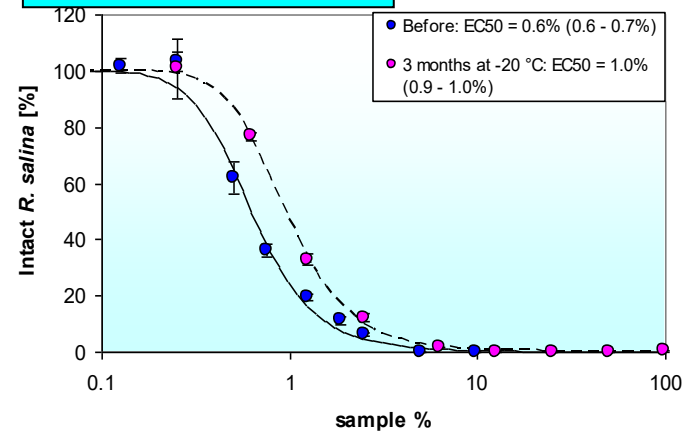
The lytic activity disappeared when stored for several days, but could be reestablished by vigorous shaking.

### Liquid-liquid extraction

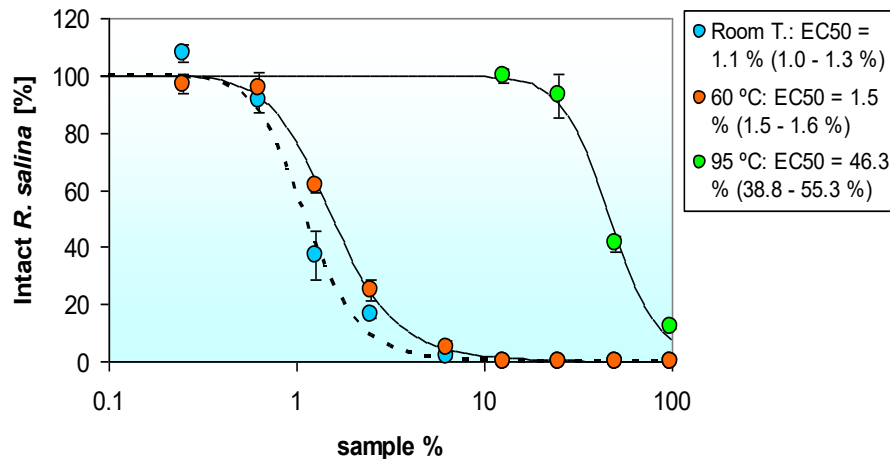
Lytic activity **cannot** be extracted by organic solvents from the aqueous supernatant, but is enriched in a foamy emulsion between the aqueous and organic phases.

## Bioactive extracellular compounds (BEC)

### Thermal stability



at -20 °C: 3 months at least  
(long term)



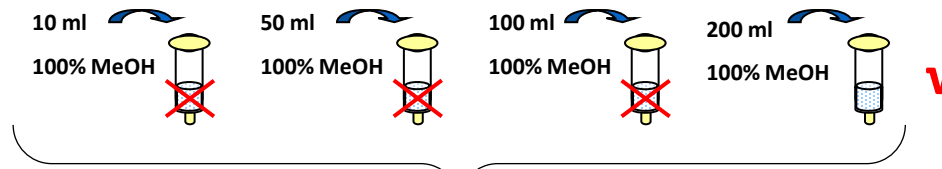
at 95 °C: 15 min  
(short term)

slight decrease at 60 °C

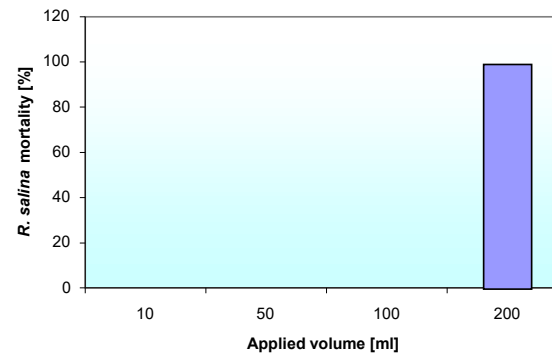
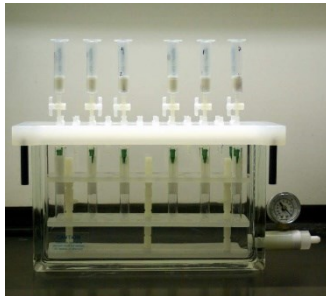
## Bioactive extracellular compounds (BEC)

### Purification: Reversed phase SPE

Alex2 supernatant



Bioassay 3h



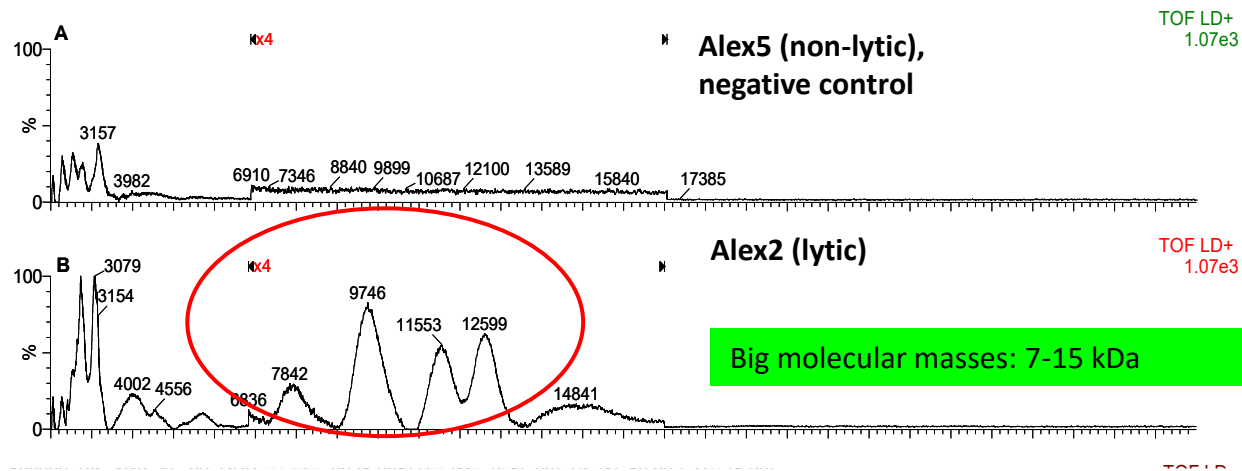
Allelochemicals „search“ their targets, i.e. specifically adsorb to lipophilic surfaces such as membranes

## Bioactive extracellular compounds (BEC)

### Mass spectrometric characterization

Molecular weight

MALDI-TOF mass spectra of active **HILIC** fraction



Ma, H., B. Krock, U. Tillmann, A. Muck, N. Wielsch, A. Svatoš, A. Cembella (2011). Harmful Algae 11: 65-72.



## Bioactive extracellular compounds (BEC)

chemical nature

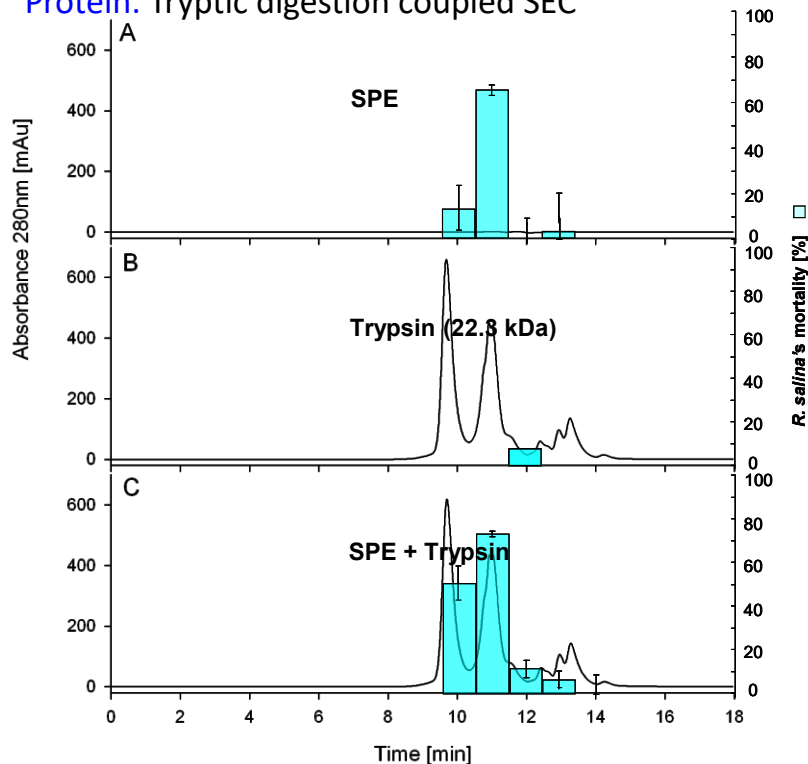
Sugar, Lipids, or protein?

**Sugar:** Phenol sulfuric acid assay

**Lipids:** n-hexane extracted supernatant (pH2)

No activity was extracted into n-hexane phase

**Protein:** Tryptic digestion coupled SEC



Unlikely to be polysaccharide...

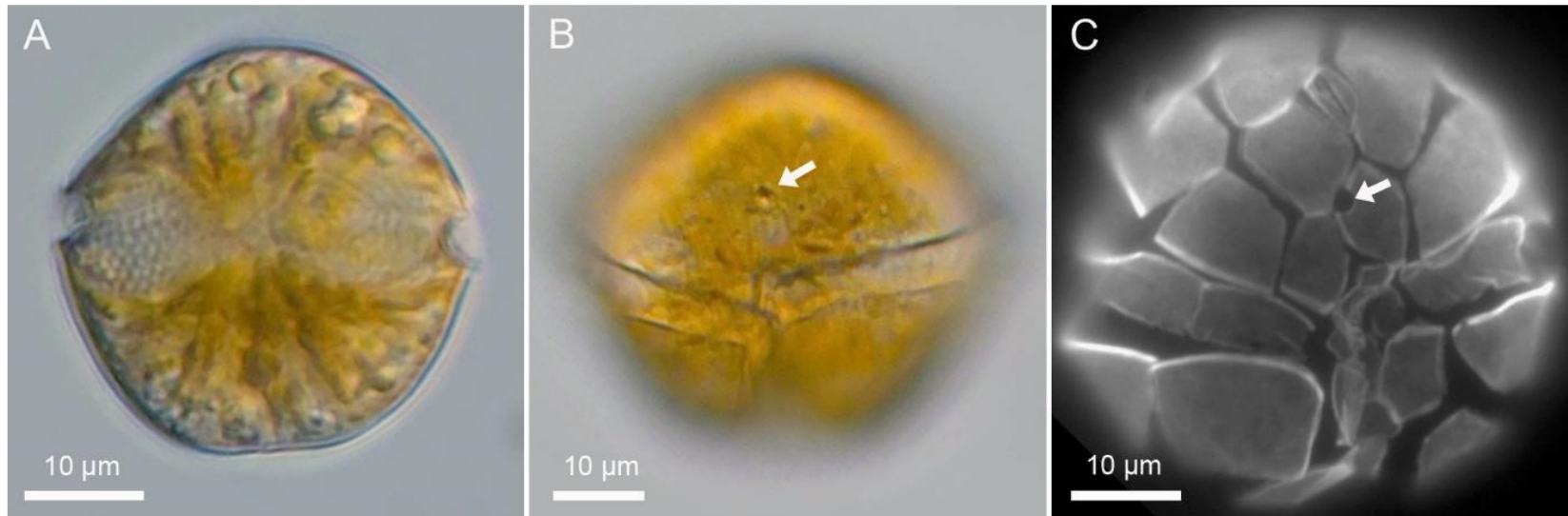
Not lipids...

Non-proteinaceous...

Large molecular smaller than  
22.3 kDa

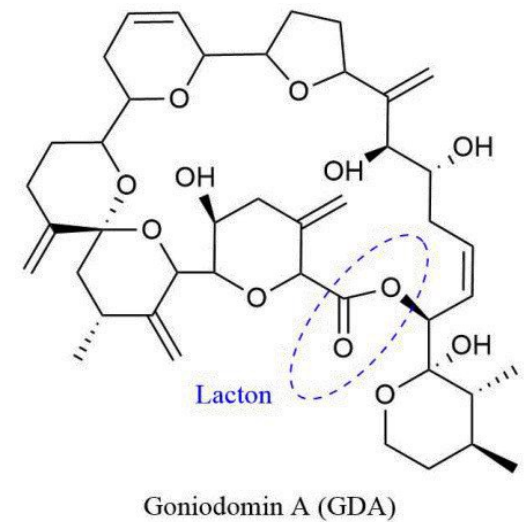
Absorbance at 280 nm and  
lytic activity of lytic SPE 80%  
methanol fraction treated  
with or without trypsin  
before applied to the  
column.

# *Alexandrium pseudogonyaulax*



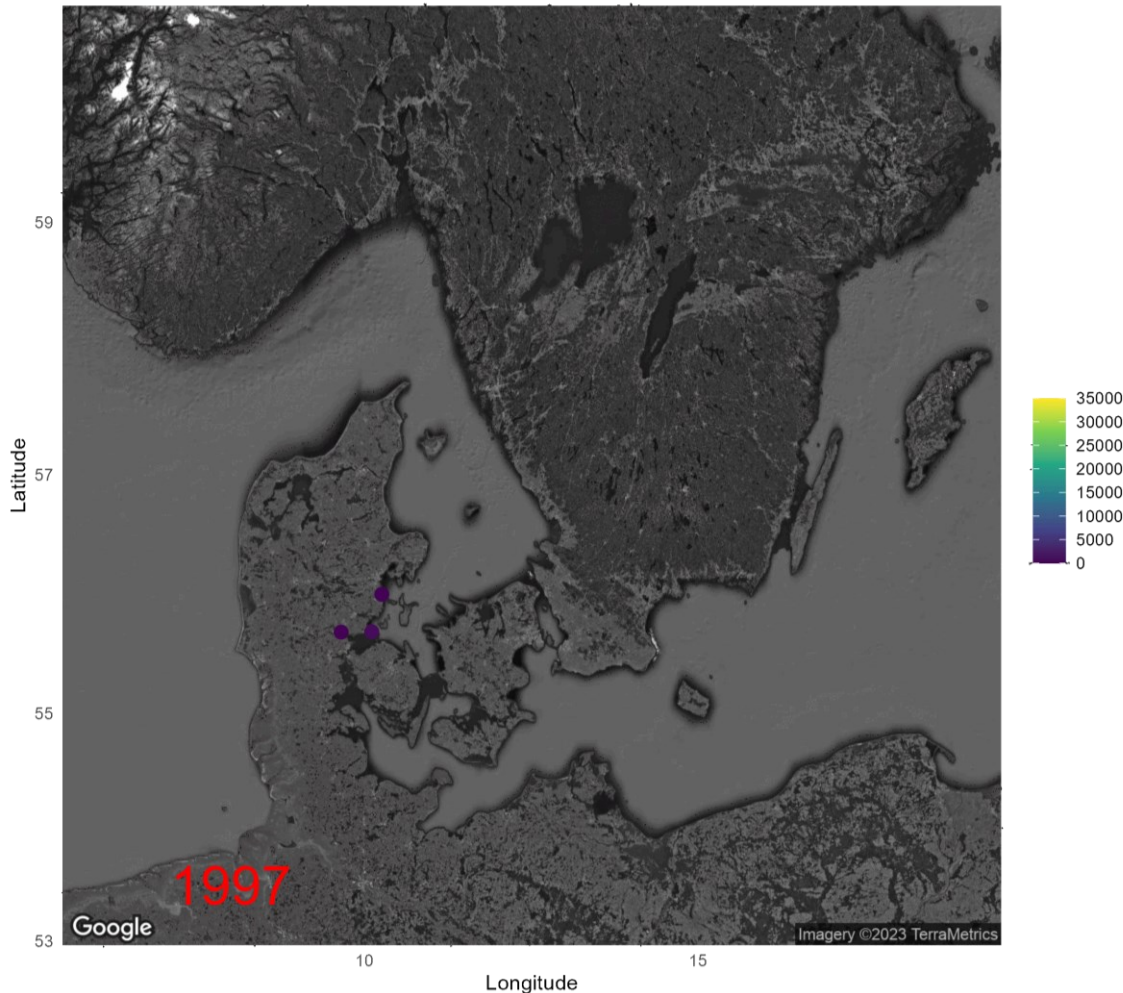
Photos: Urban Tillmann

- Thecate dinoflagellate
- Producer of goniodomins (GDs)
- Producer of bioactive extracellular compounds (BEC)
- Suspected to be ichthyotoxic



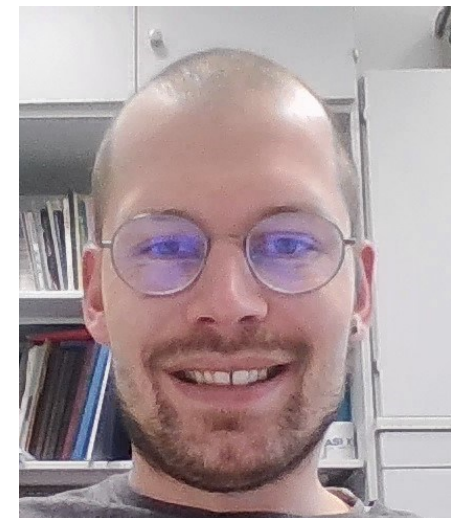
# Alexandrium pseudogonyaulax

Expansion of *A. pseudogonyaulax*



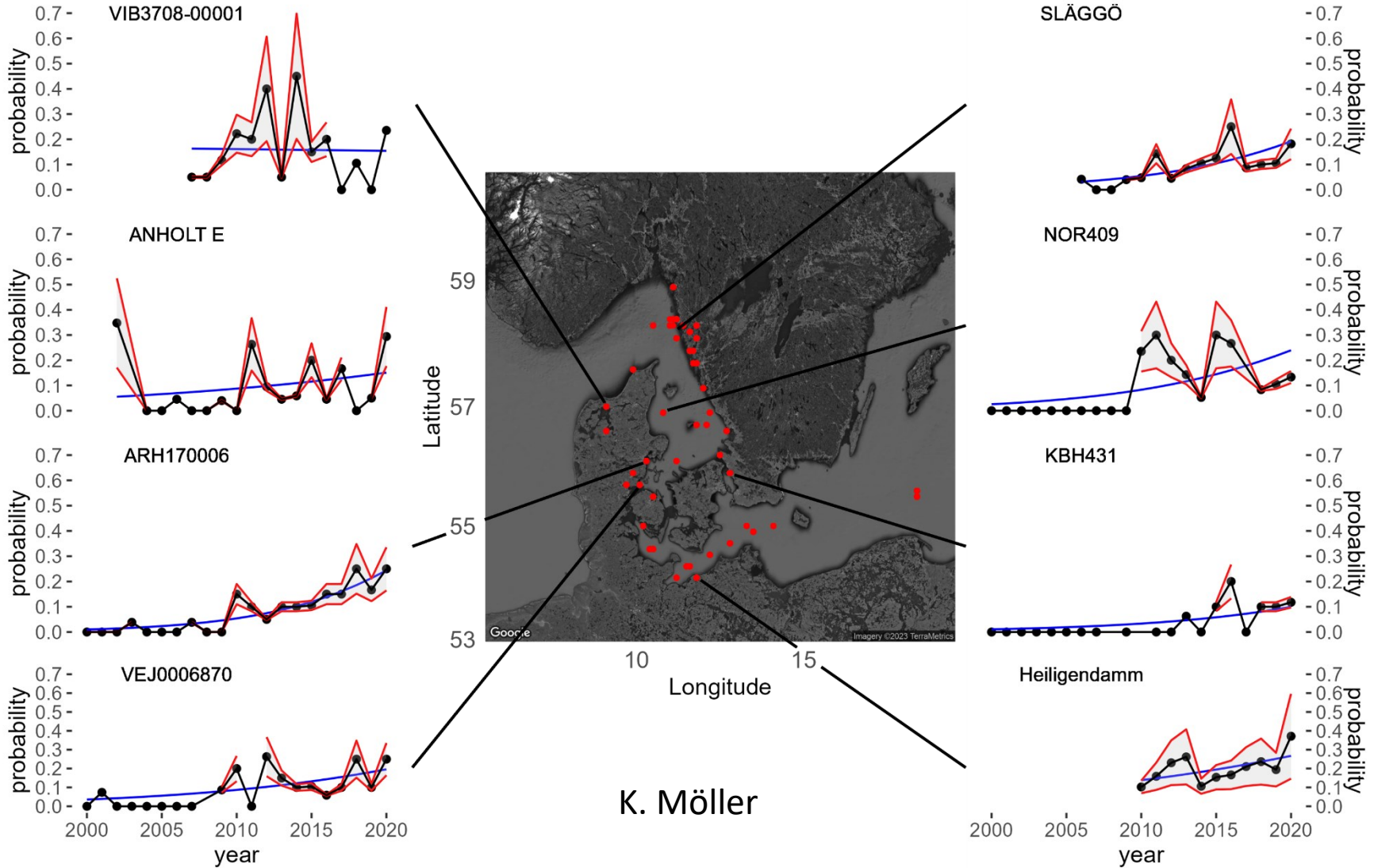
PhD thesis:

Causes of the spread of the harmful and potentially fish-toxic microalgae *Alexandrium pseudogonyaulax* (Dinophyceae) in German coastal waters and assessment of the future risk potential



Kristof Möller

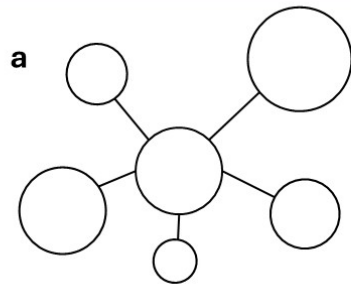
# Alexandrium pseudogonyaulax



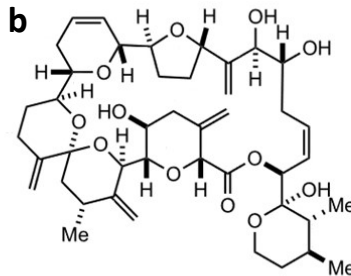


# Alexandrium pseudogonyaulax

## Background:

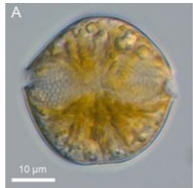


unknown Bioactive  
Extracellular Compounds  
(BECs)



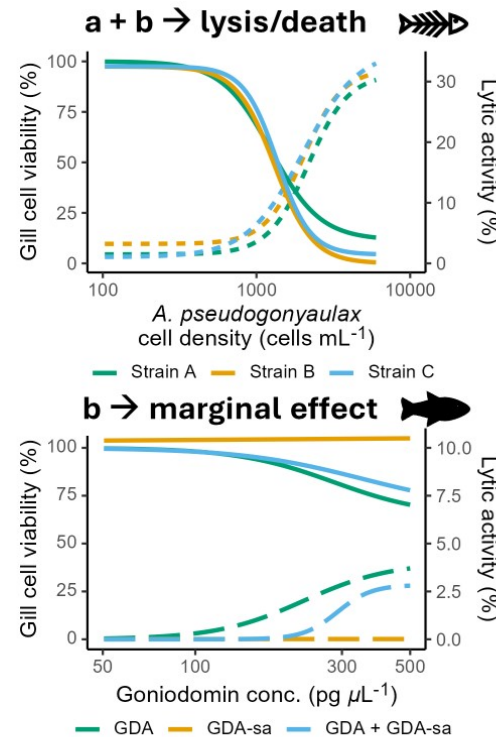
Goniodomins  
(Goniodomin A)

*Alexandrium  
pseudogonyaulax*

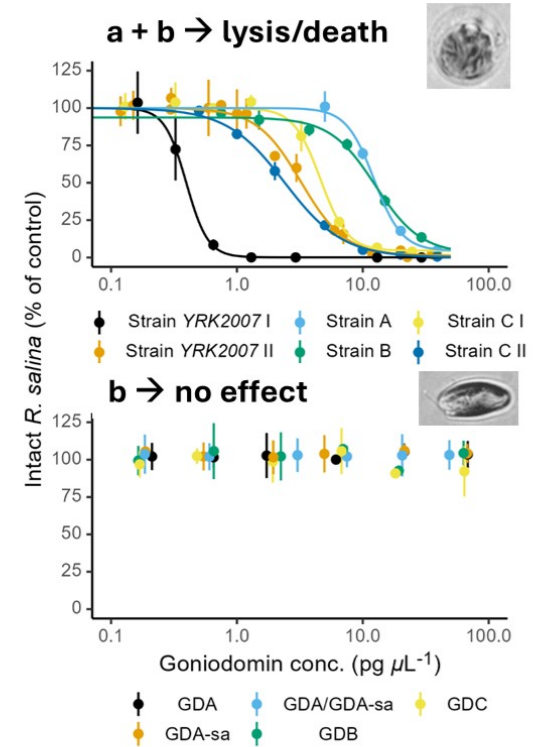


## Results:

Bioassays Rtgill-W1 (fish cells)



Bioassays *R. salina* (microalgae)



**Conclusion: Toxic effects of *Alexandrium pseudogonyaulax* are likely driven by BECs and not by goniodomins**

K. Möller

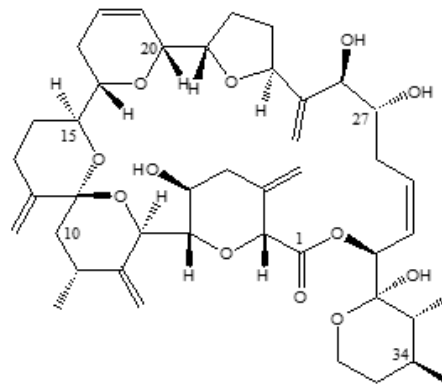
**Organism**

**Chemical compound**

**Ecological Function**

## Goniodomins

*Alexandrium  
pseudogonyaulax*



?

*Alexandrium  
pseudogonyaulax*

?

Defense against Predators

Elimination of Competitors

Bioactive Extracellular Compound  
(BEC)



Any  
Questions?

Thanks for  
Your Attention!