



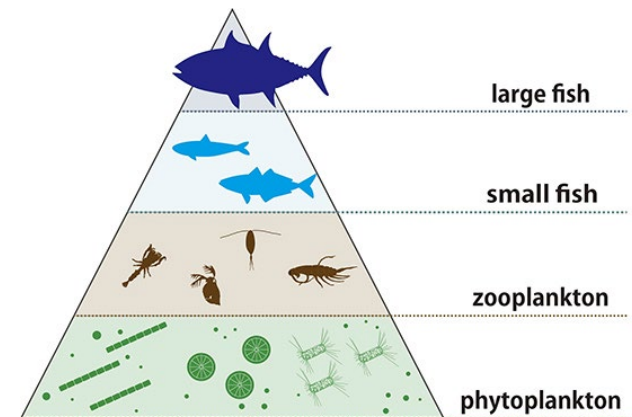
What do we know about ichthyotoxic microalgal species and their toxins?

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

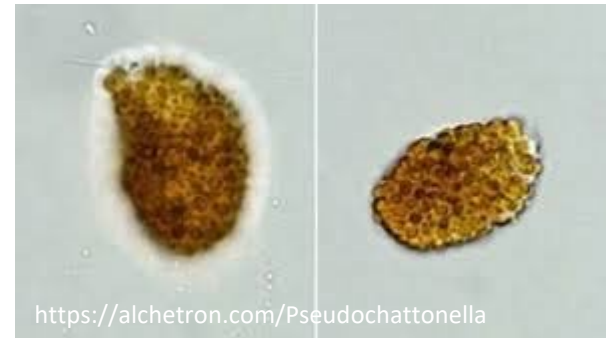
April/May 2016, Región de Los Lagos: massive bloom event of *Alexandrium catenella* associated with high shellfish contamination



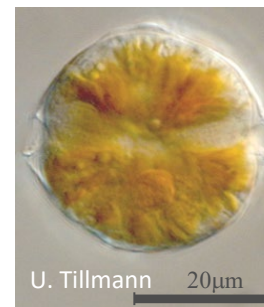
Both blooms were almost coinciding in time and space and caused mass mortality of marine life, especially in salmon aquaculture

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.

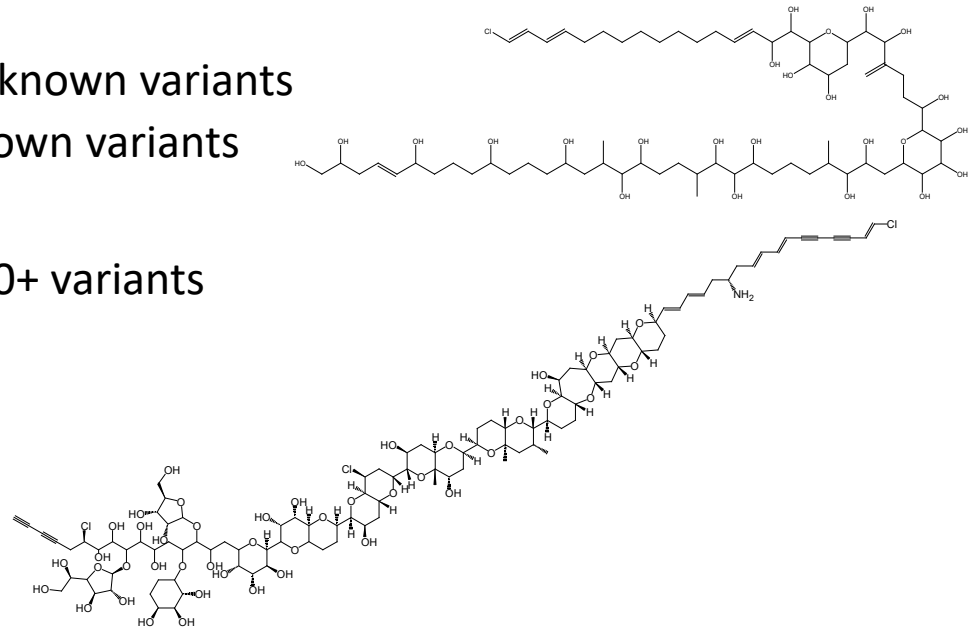
Chrysocromulina spp.

Fibrocapsa japonica

Heterosigma akashiwo

Protoceratium reticulatum

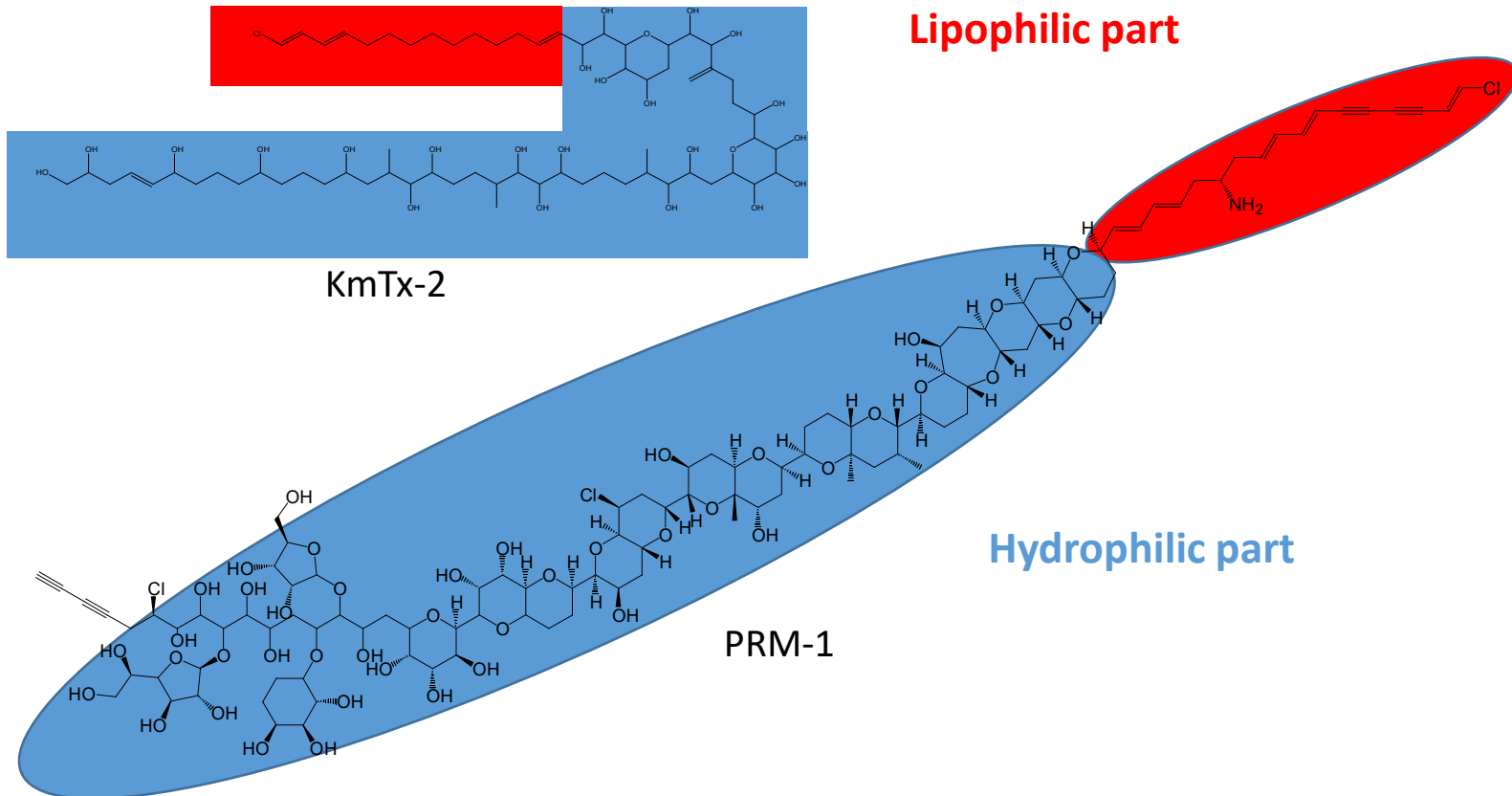
Pseudochattonella cf. *verruculosa*



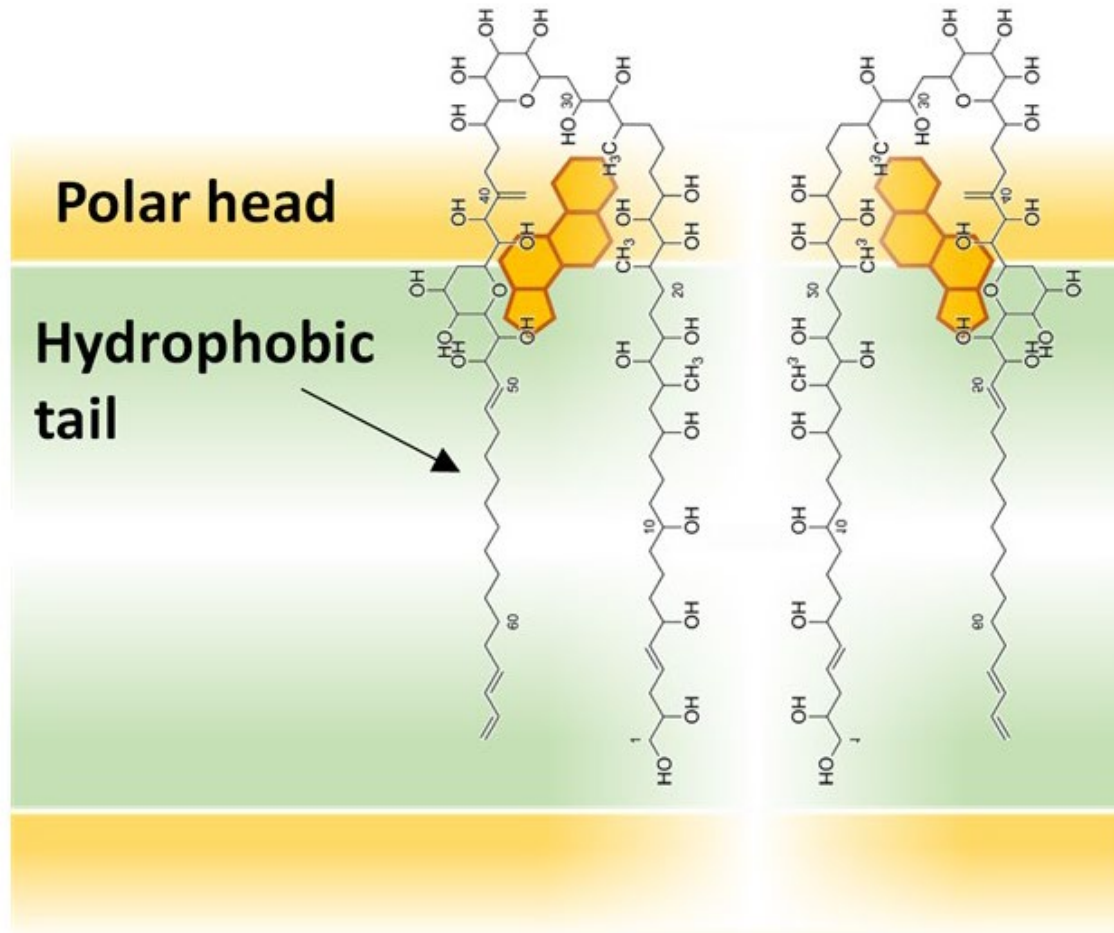
Ichthyotoxins:

Unknown !!

Known Ichthyotoxins



Mode of action

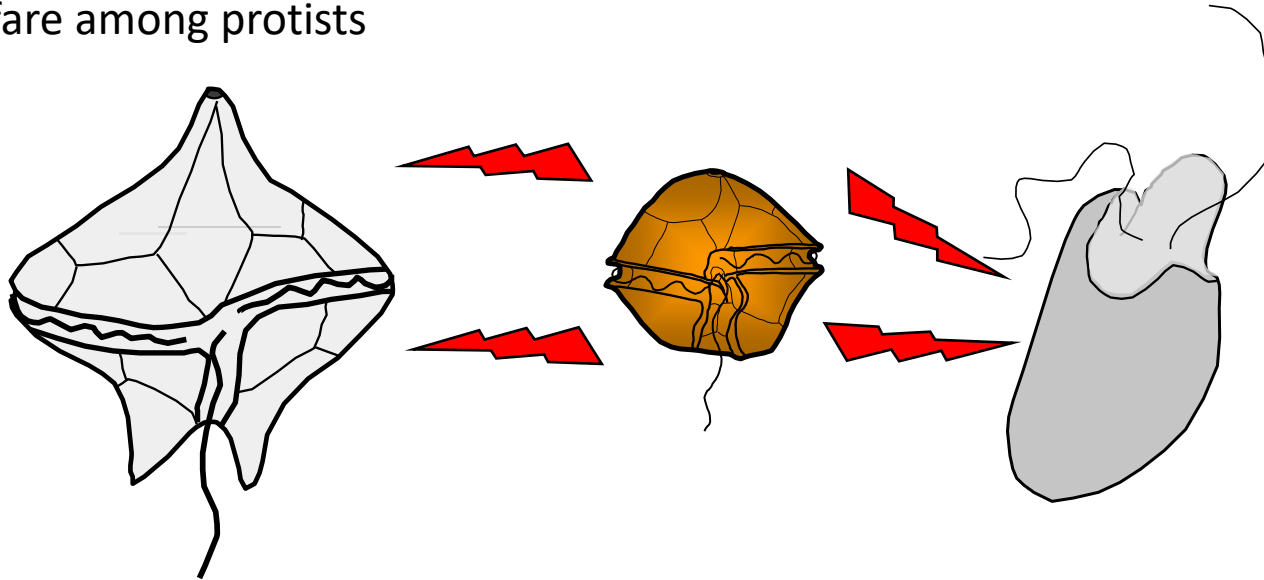


Long, M., et al. (2021) *Toxins* 13(12): 905.

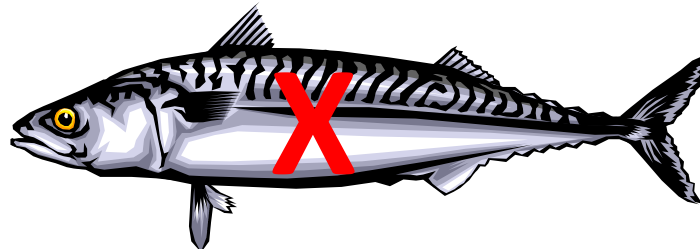
Ichthyotoxins

Current Hypothesis:

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



Ichthyotoxicity is a collateral damage of protistan allelochemistry



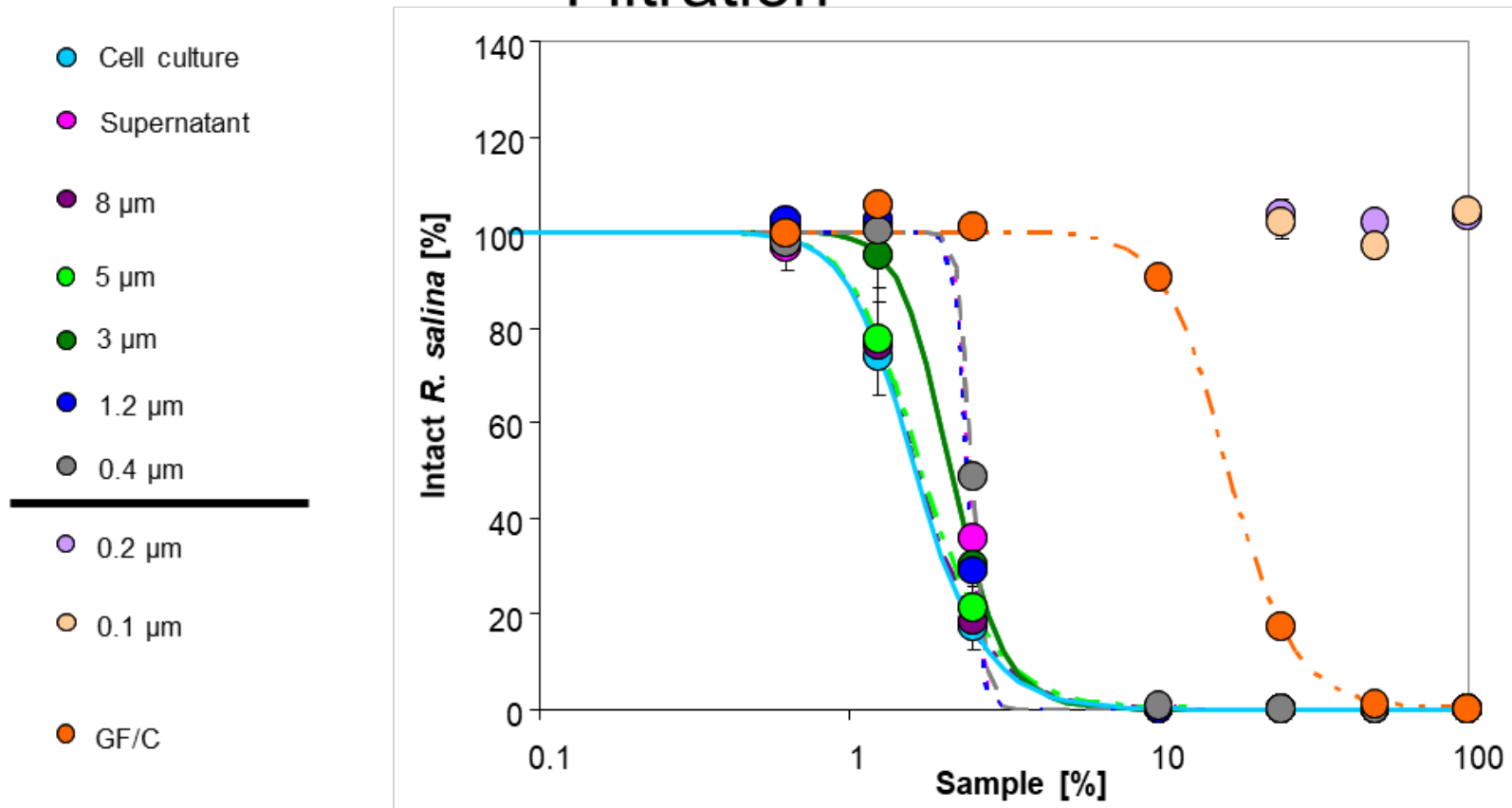
Alexandrium catenella

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



Bioactive extracellular compounds (BEC)

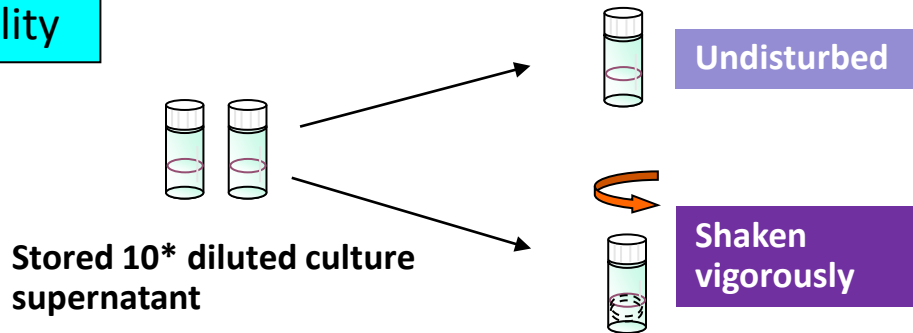
Filtration



Alexandrium catenella

Bioactive extracellular compounds (BEC)

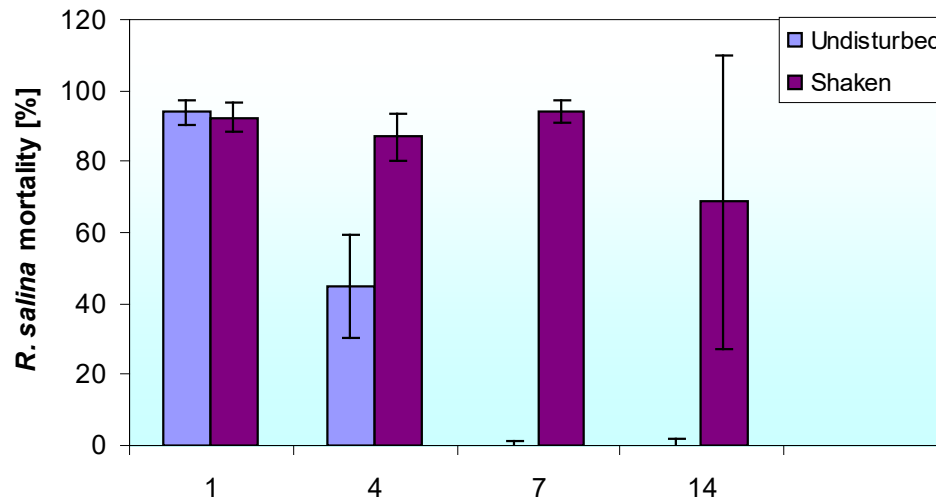
Stability



Liquid-liquid extraction

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

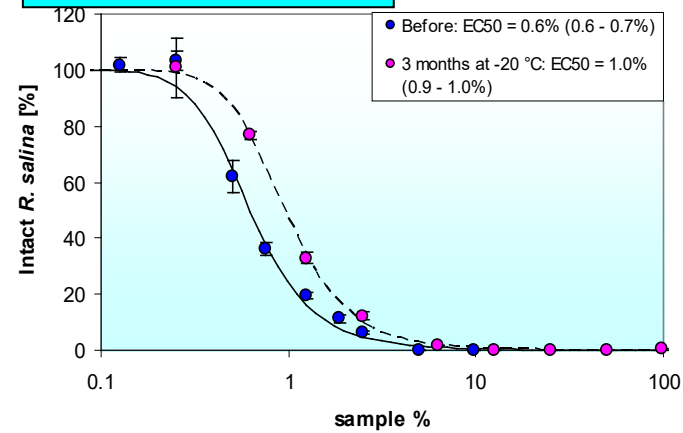
but is enriched in a foamy emulsion between the aqueous and organic phases.



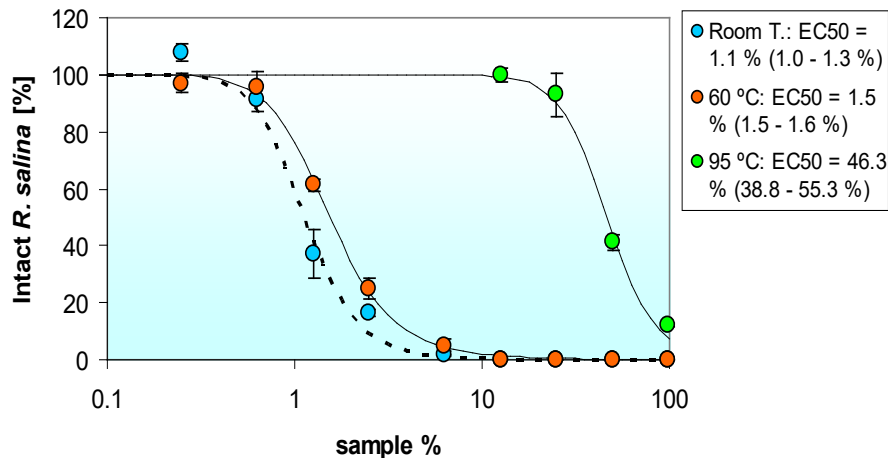
The lytic activity disappeared when stored for several days, but could be reestablished by vigorous shaking.

Bioactive extracellular compounds (BEC)

Thermal stability



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



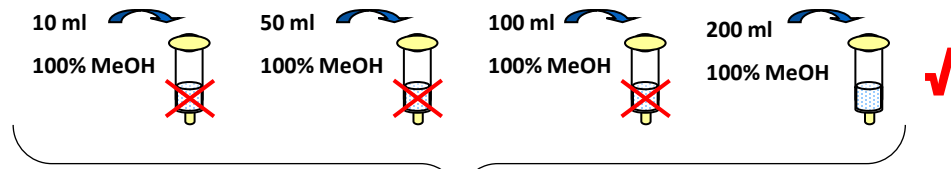
at 95 °C: 15 min
(short term)

slight decrease at 60 °C

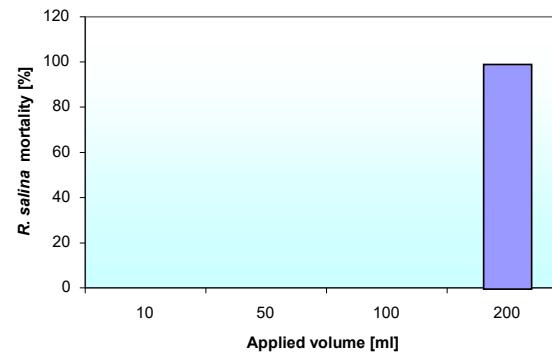
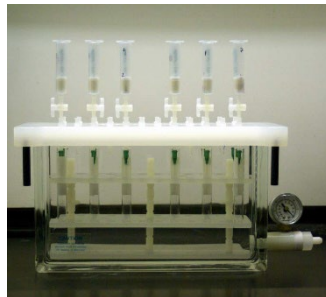
Bioactive extracellular compounds (BEC)

Purification: Reversed phase SPE

Alex2 supernatant



Bioassay 3h



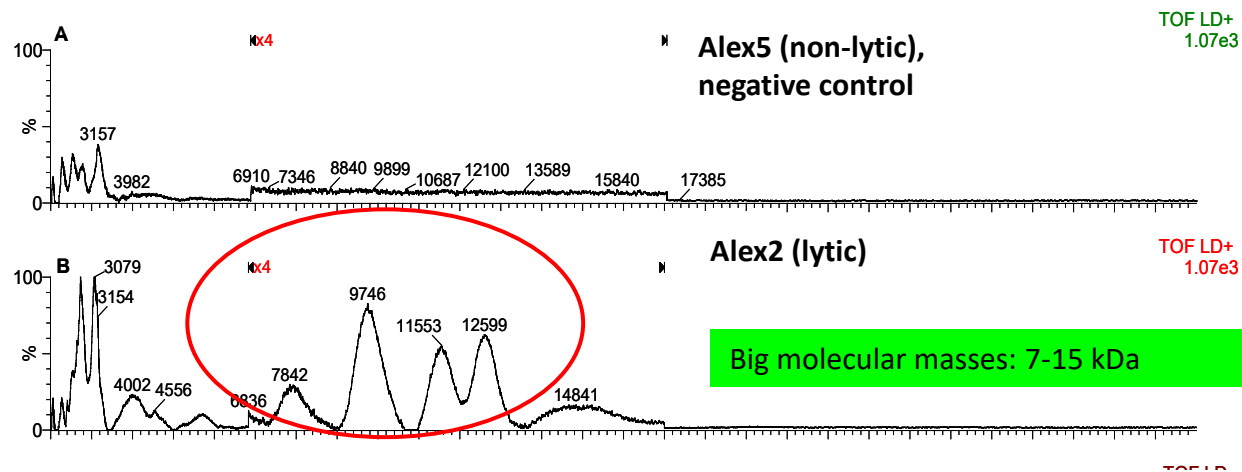
Allelochemicals „search“ their targets, i.e. specifically adsorb to lipophilic surfaces such as membranes

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

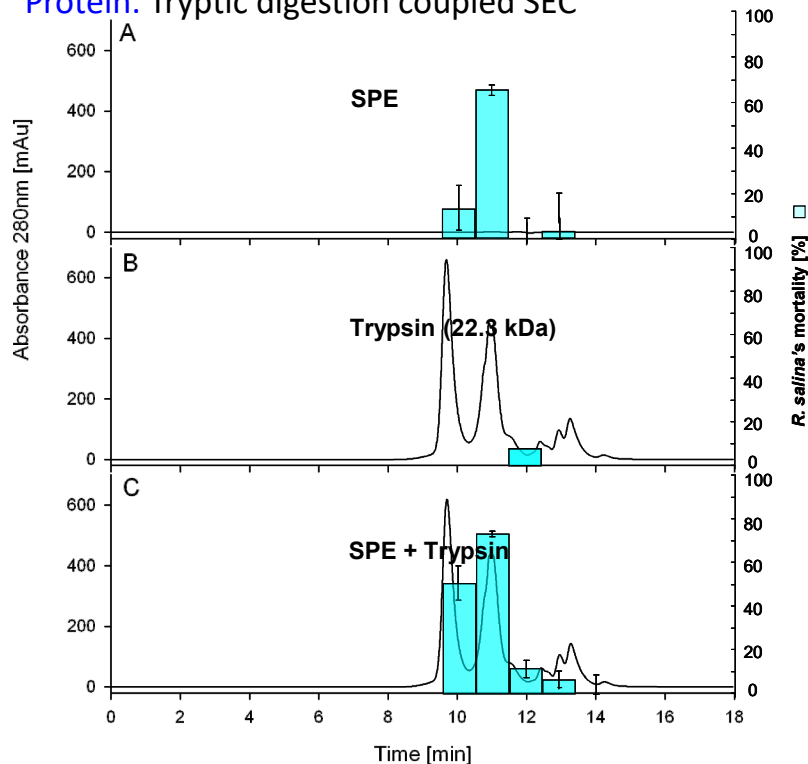
Sugar, Lipids, or protein?

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...

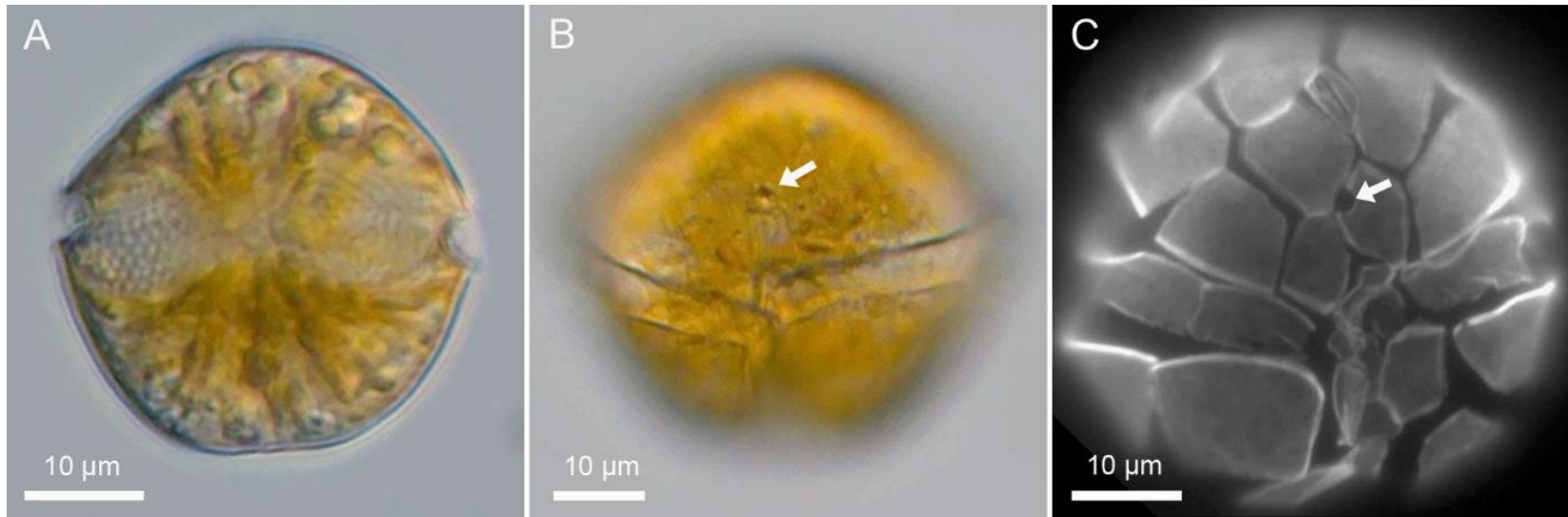
Not lipids...

Non-proteinaceous...

Large molecular smaller than
22.3 kDa

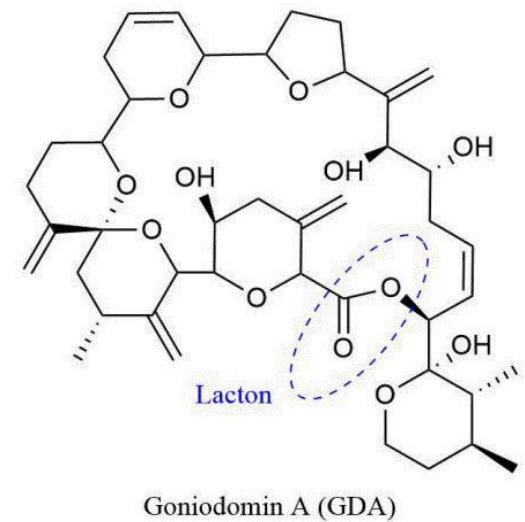
Absorbance at 280 nm and
lytic activity of lytic SPE 80%
methanol fraction treated
with or without trypsin
before applied to the
column.

Alexandrium pseudogonyaulax



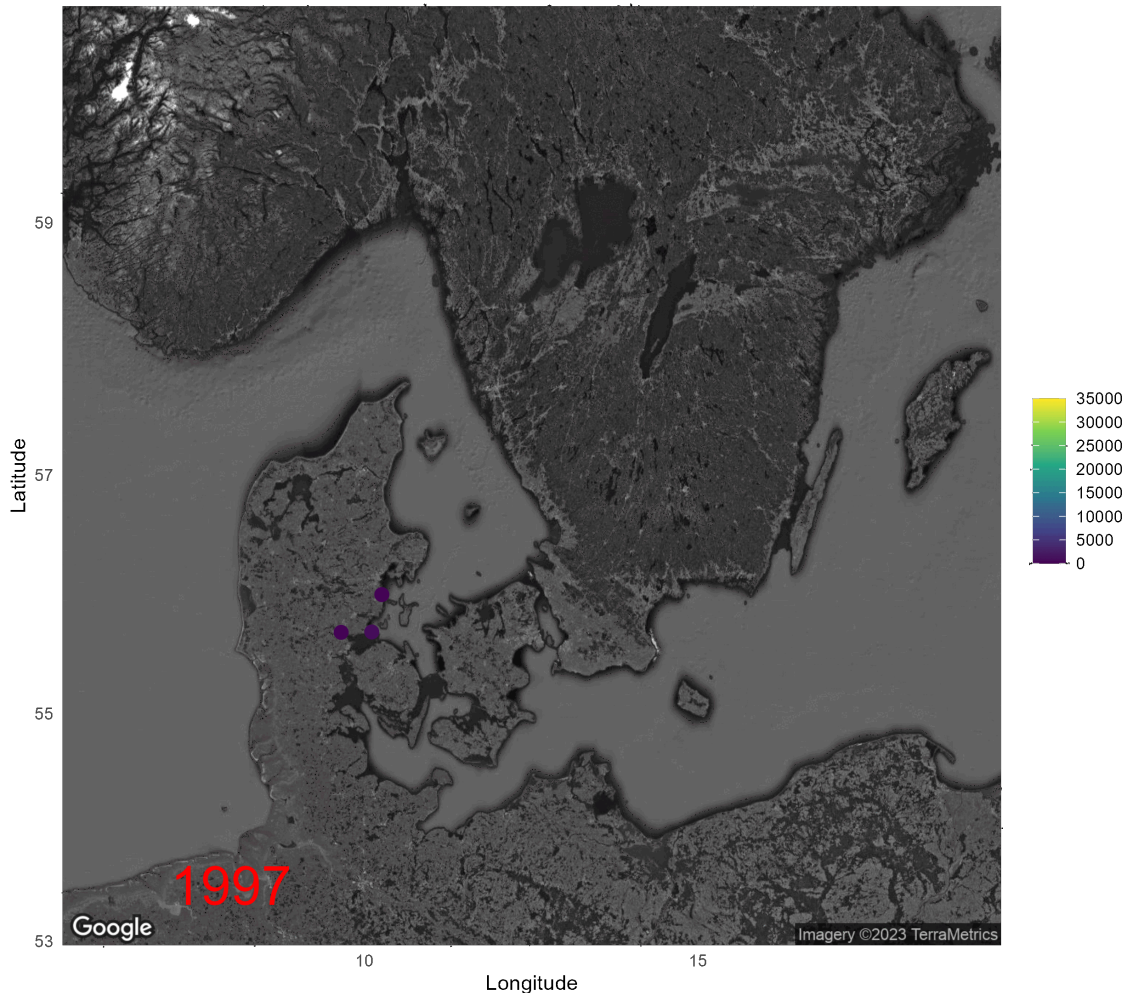
Photos: Urban Tillmann

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



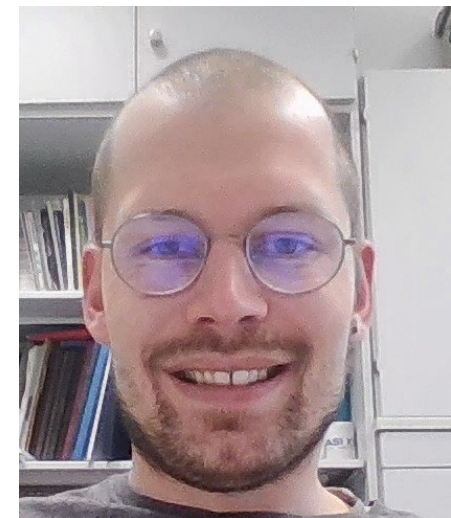
Alexandrium pseudogonyaulax

Expansion of *A. pseudogonyaulax*



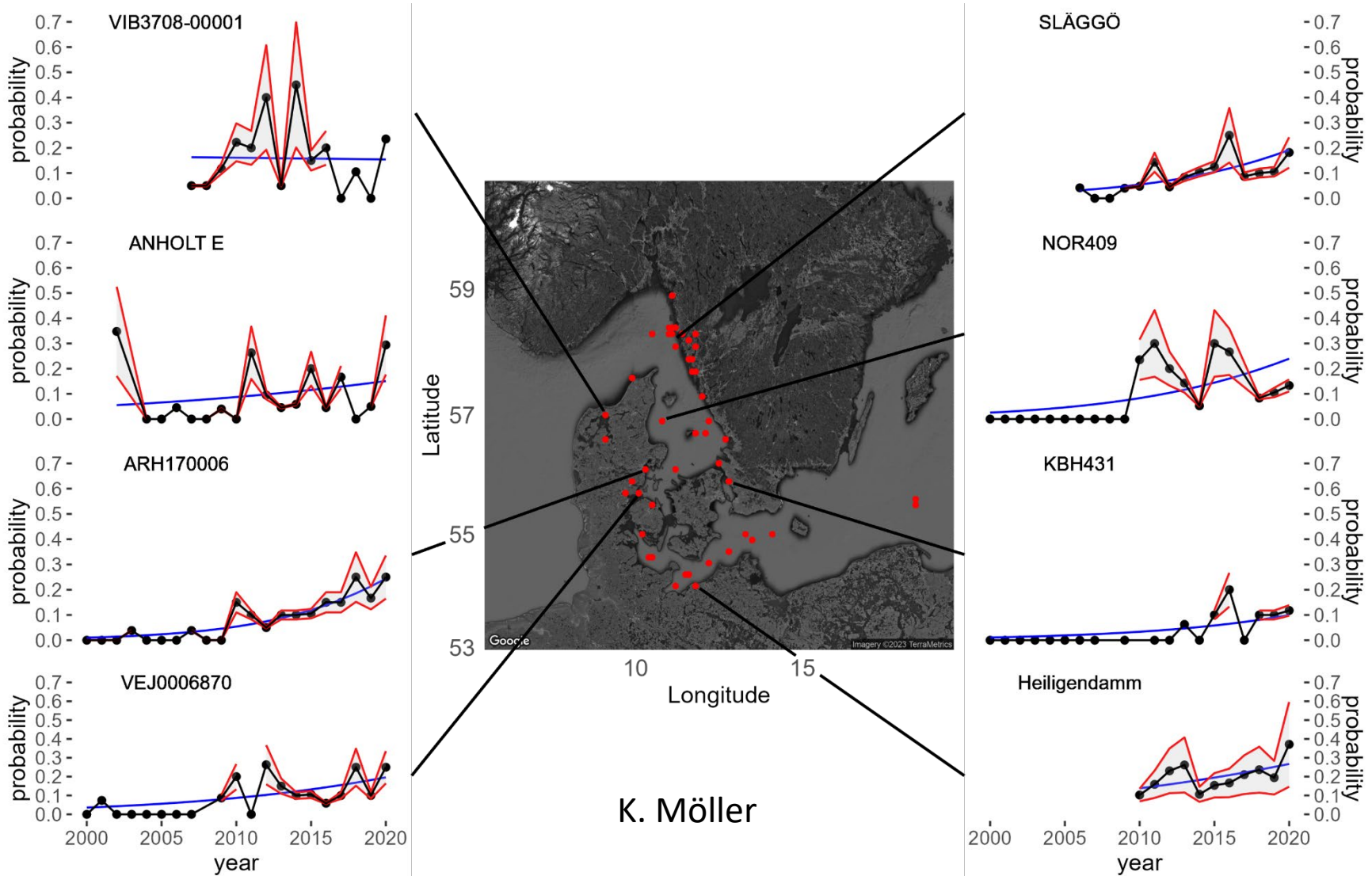
PhD thesis:

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



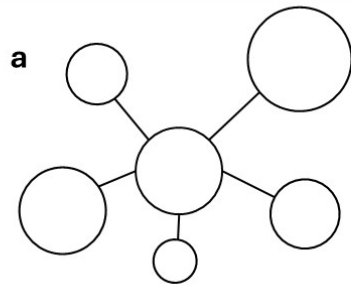
Kristof Möller

Alexandrium pseudogonyaulax

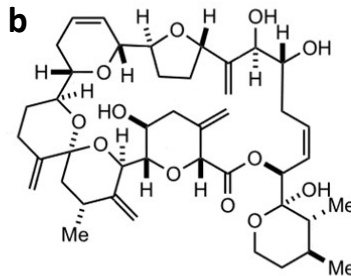


Alexandrium pseudogonyaulax

Background:

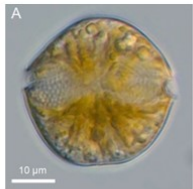


unknown Bioactive
Extracellular Compounds
(BECs)



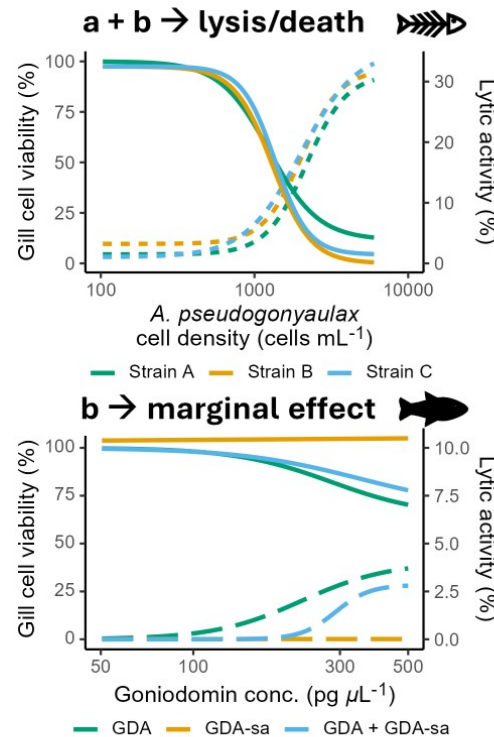
Goniodomins
(Goniodomin A)

*Alexandrium
pseudogonyaulax*

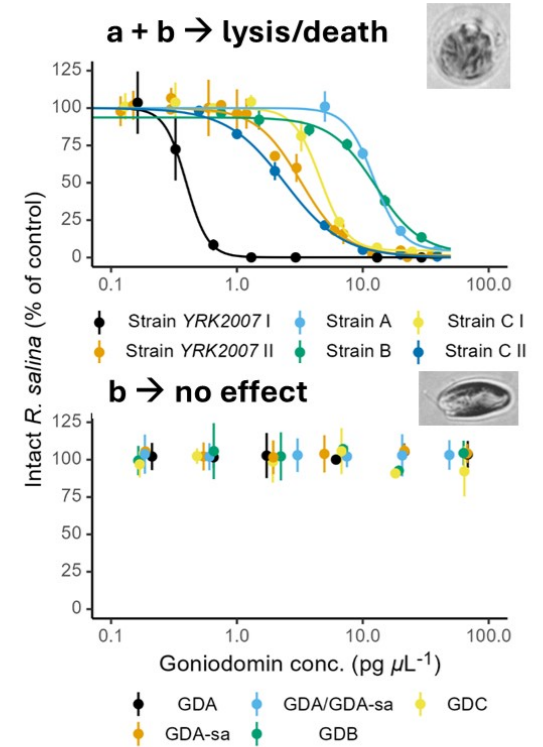


Results:

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

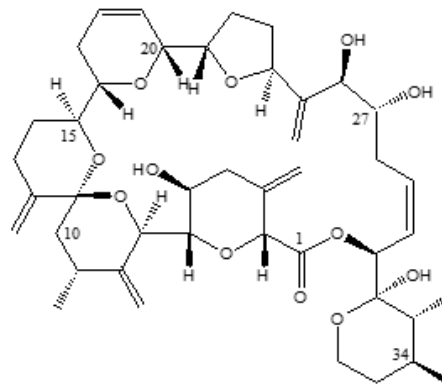
Organism

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!