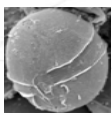


# Revealing the Allelochemical Potential of *Alexandrium ostenfeldii*, a Marine Dinoflagellate

Bernd Krock, Urban Tillmann, Uwe John, Nina Jaekisch,  
Allan D. Cembella



# *Alexandrium ostenfeldii*

Marine gonyaulacoid mixotroph dinoflagellate in temperate waters



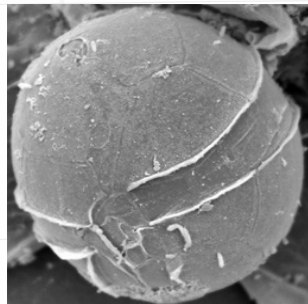
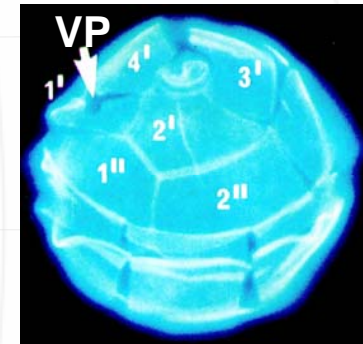
## **Ocurrence:**

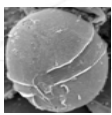
Atlantic (Canada, USA, Iceland, Faroe Islands, Spain)

North Sea (Scotland, Norway, Denmark)

Mediterranean Sea (Italy, Egypt)

Pacific (USA, Russia, New Zealand)





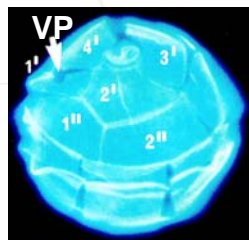
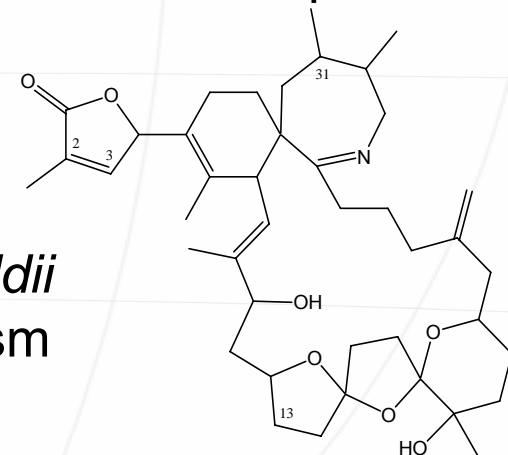
## A. *Ostenfeldii* – Short History

1904: *A. ostenfeldii* first described as *Goniodoma ostenfeldii* by Paulsen in Iceland in 1904

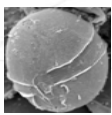


July 1990, 1991: Unusual mouse-deaths - lipophilic mussel extracts from Ship Harbour, Nova Scotia, Canada: “fast acting toxin” (FAT) symptoms and coincident consumer complaints of mild illness after shellfish consumption

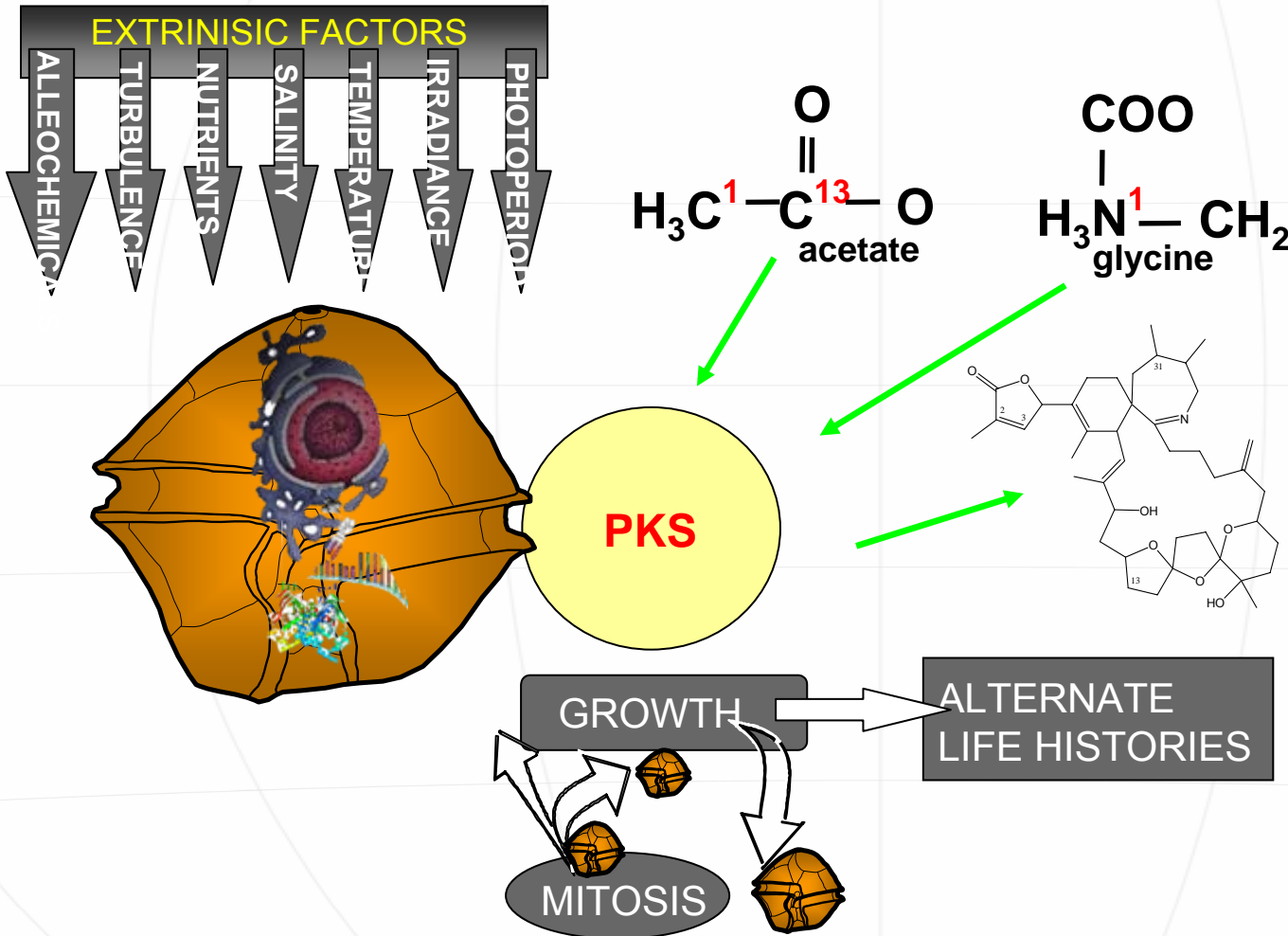
1995: Structural elucidation of spiroolides



1996: *Identification of A. ostenfeldii* as spirolide producing organism



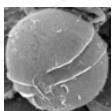
# Regulation of Spirolide Biosynthesis



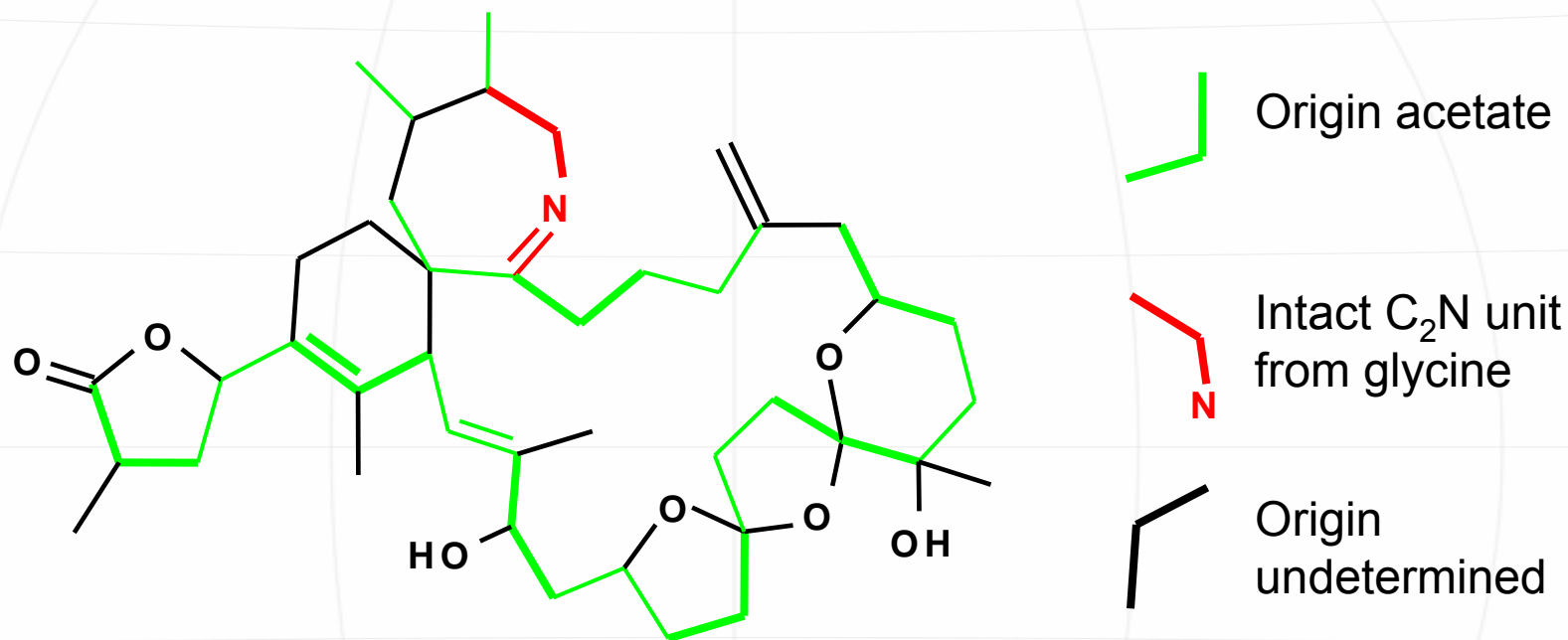
Toxin production is related to extrinsic factors

Toxin composition is maintained, presumably genetically determined

Effect of grazing pressure and competition with other microalgae needs further investigation

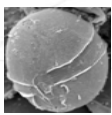


# Stable Isotope Feeding of *A. ostentfeldii*



carbon skeleton is produced by polyketide synthases (PKS) out of acetate units

13-desmethyl Spirolide C is a polyketide derived compound



# Genetic Analysis

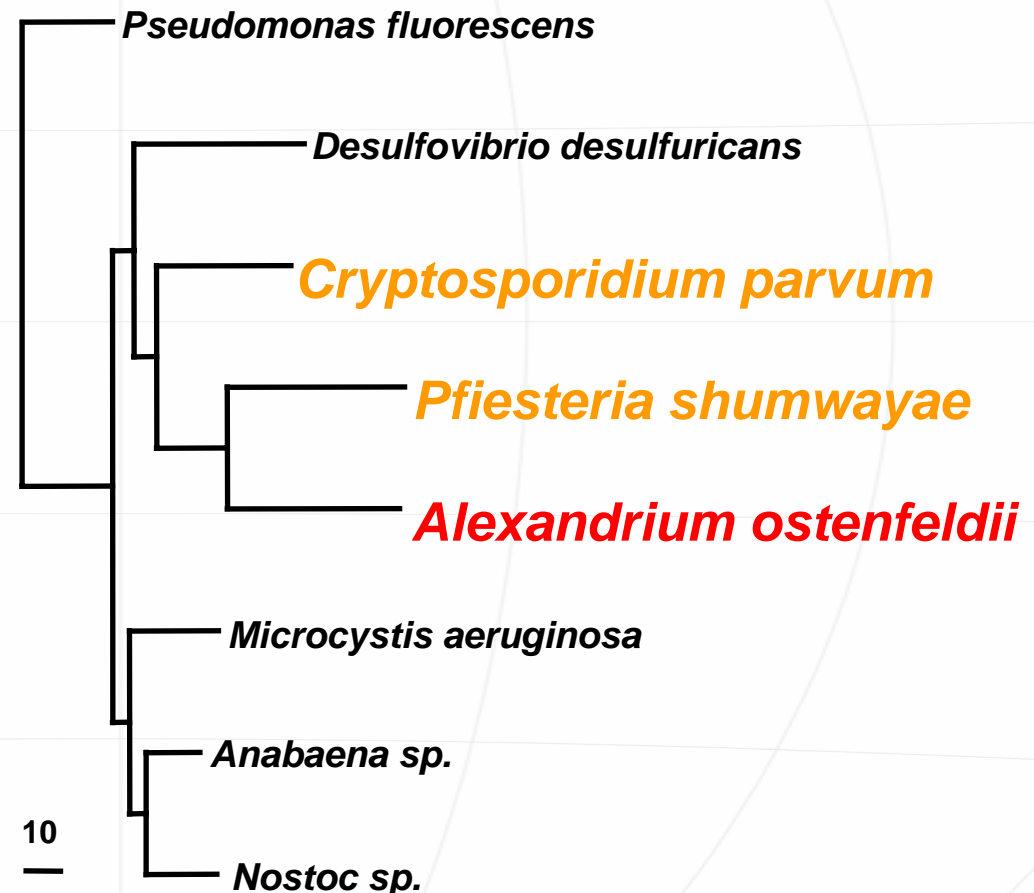
Generation of a normalized cDNA library of *A. ostensfeldii*.

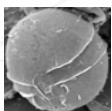
Sequencing of approx. 5000 clones.

Successful attribution of 15% of the clones to gene functions of almost all expected functional categories.

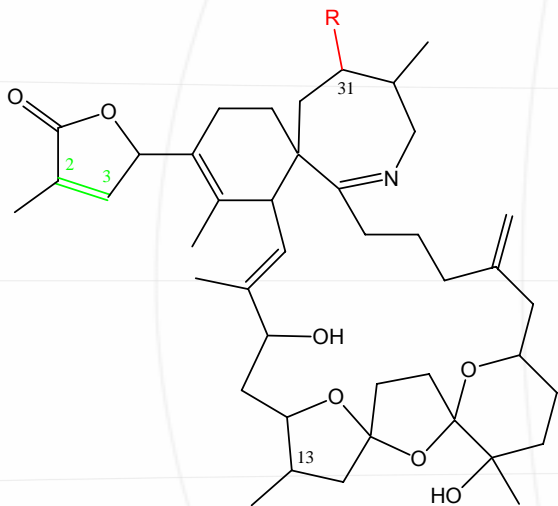
Identification of eight genes related to stress, defence and toxicity (putative PKS genes).

## PKS EST Analysis

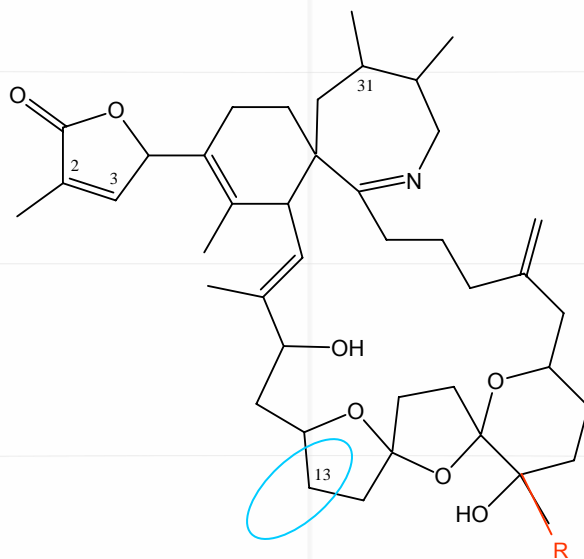




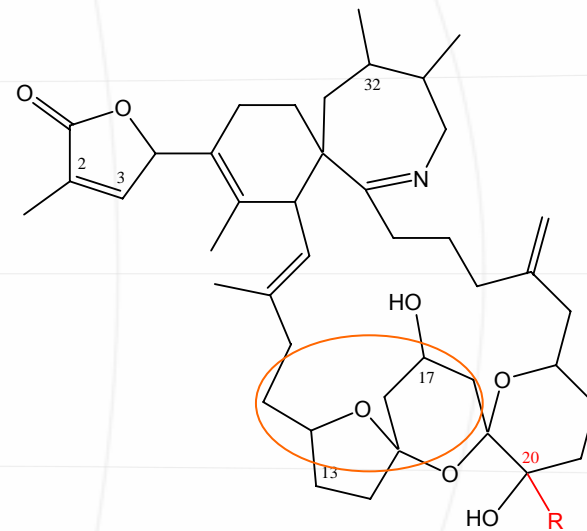
# Spirolide Variability



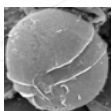
Spirolide A:  $R = H$ ,  $\Delta^{2,3}$   
B:  $R = H$   
C:  $R = Me$ ,  $\Delta^{2,3}$   
D:  $R = Me$



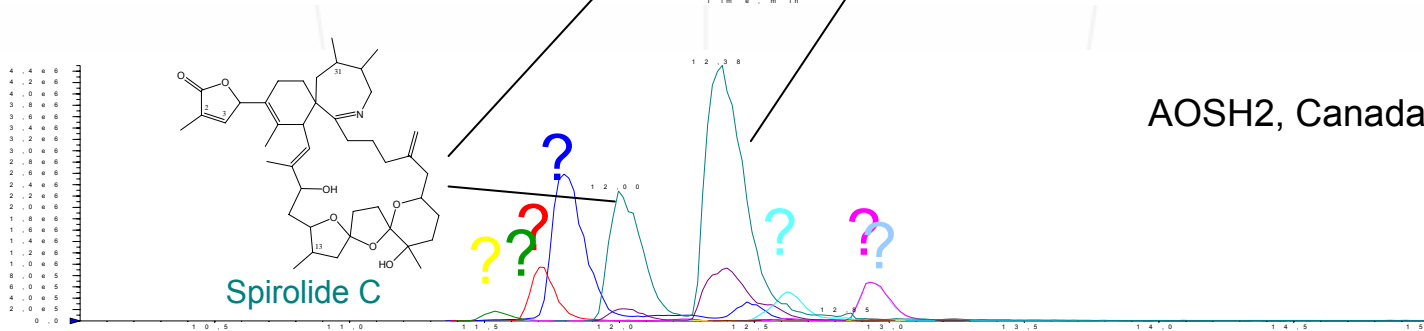
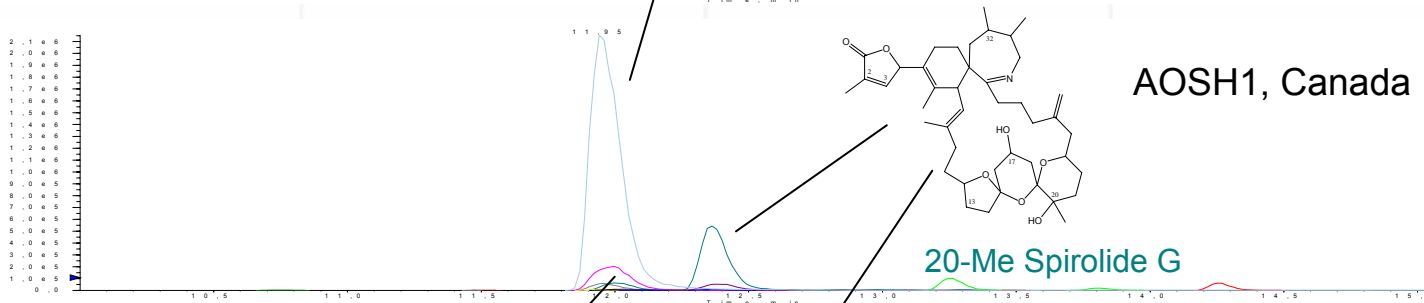
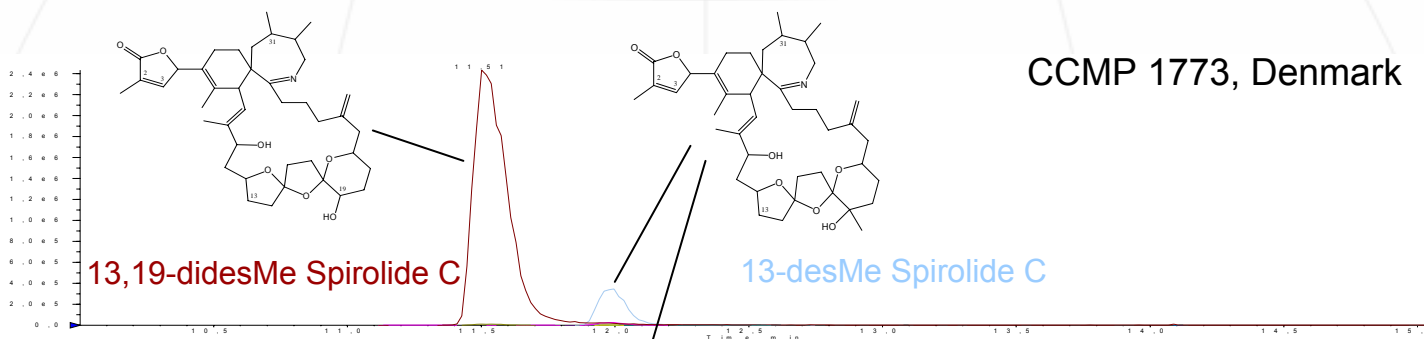
13-DesMe Spirolide C:  $R = Me$   
13,19-DidesMe Spirolide C:  $R = H$



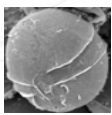
Spirolide G:  $R = H$   
20-Me Spirolide G:  $R = Me$



# Spirolide Variability







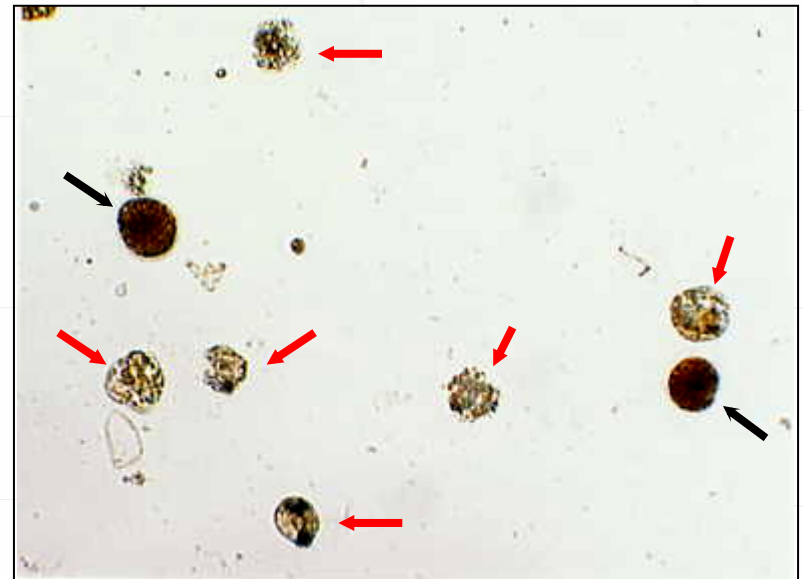
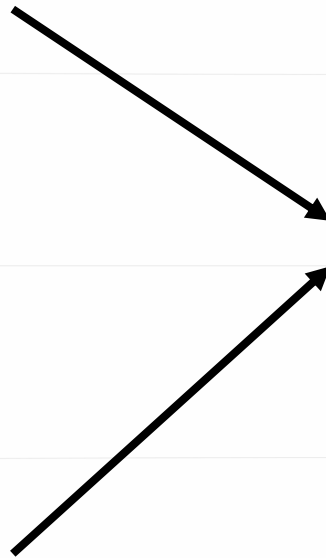
## Lytic Effect of *A. ostenfeldii*



*Oxyrrhis marina*



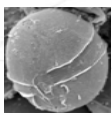
*Alexandrium ostenfeldii*



Lytic Effect of *Alexandrium* shown with *Oxyrrhis marina*.

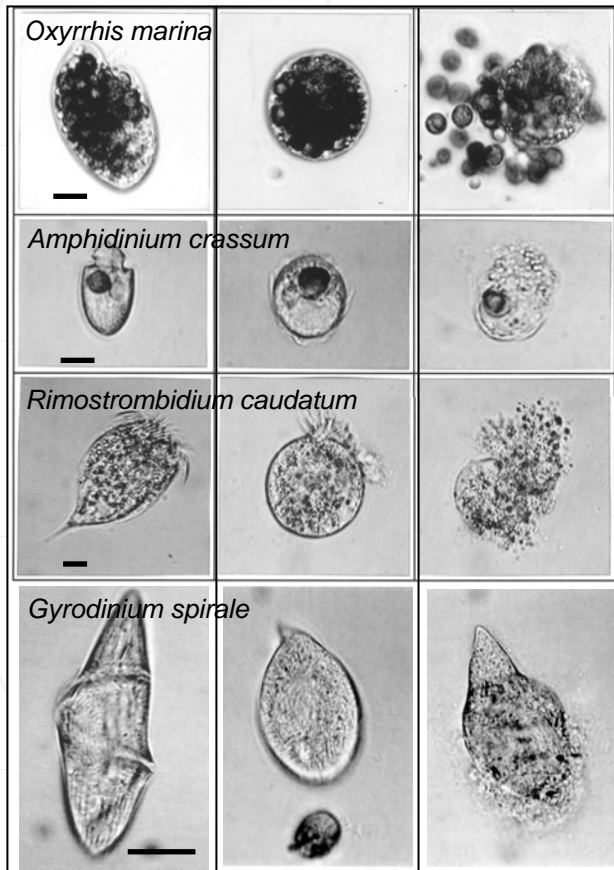
Black arrows: *Alexandrium*  
Red arrows: Reminders of *Oxyrrhis*

Photos: U. Tillmann



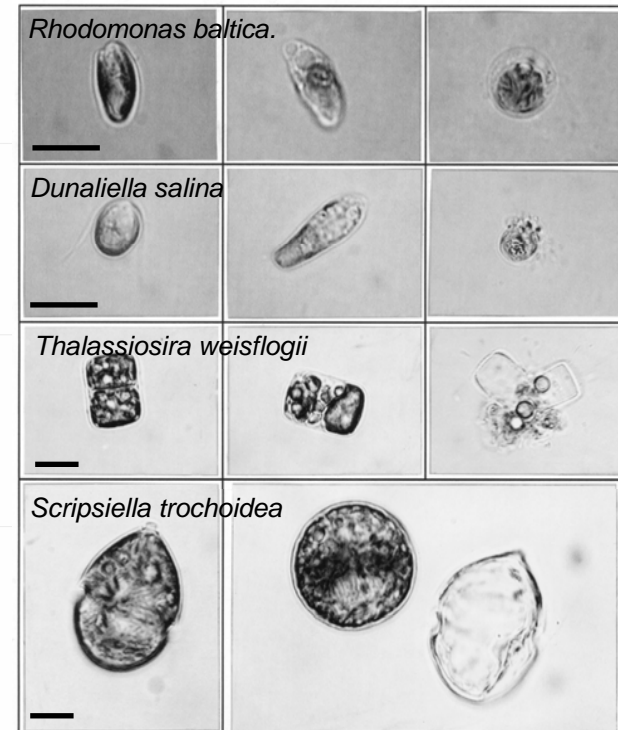
# Lytic Effect of *A. ostenfeldii*

## Heterotrophs

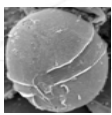


Scale bar = 10  $\mu$ m

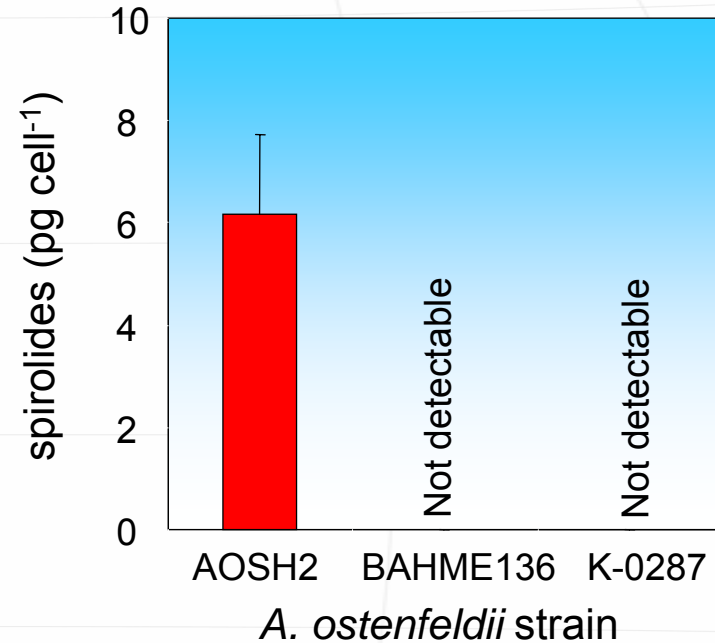
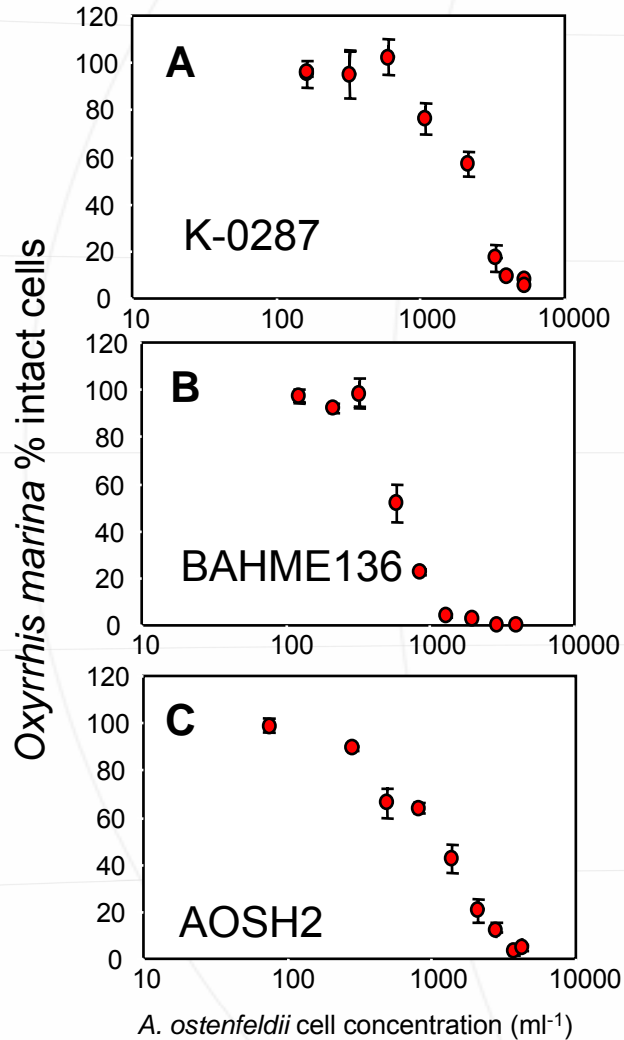
## Autotrophs



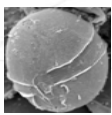
Microscopical observations of *Alexandrium* lytic effects on different target species



# Lytic Effect of *A. ostenfeldii*



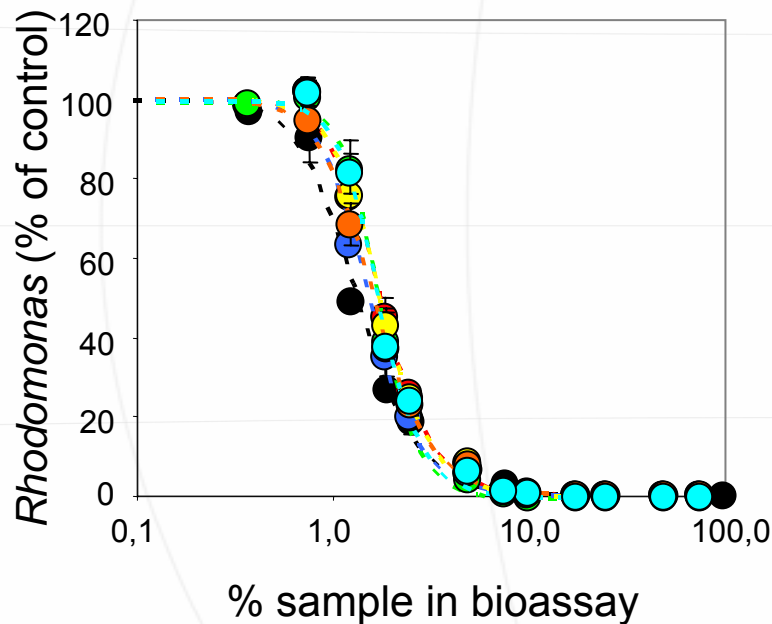
**Allelochemical potency is not related to spirolide production**



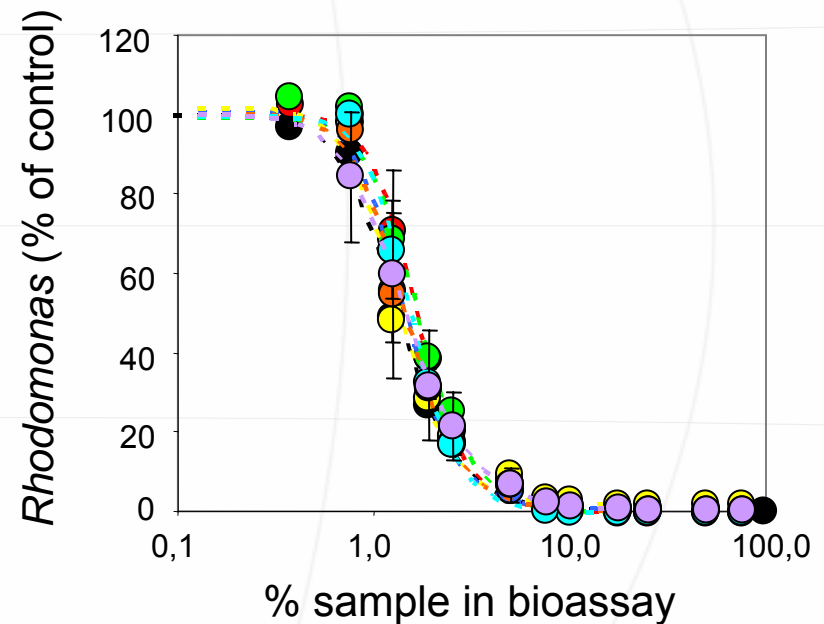
# Lytic Activity of Extracellular Compounds – Stability

*Alexandrium tamarense* supernatant – Lytic Effect on *Rhodomonas*

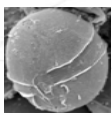
15°C; light (150  $\mu\text{E m}^{-2} \text{s}^{-1}$ )



15°C; dark

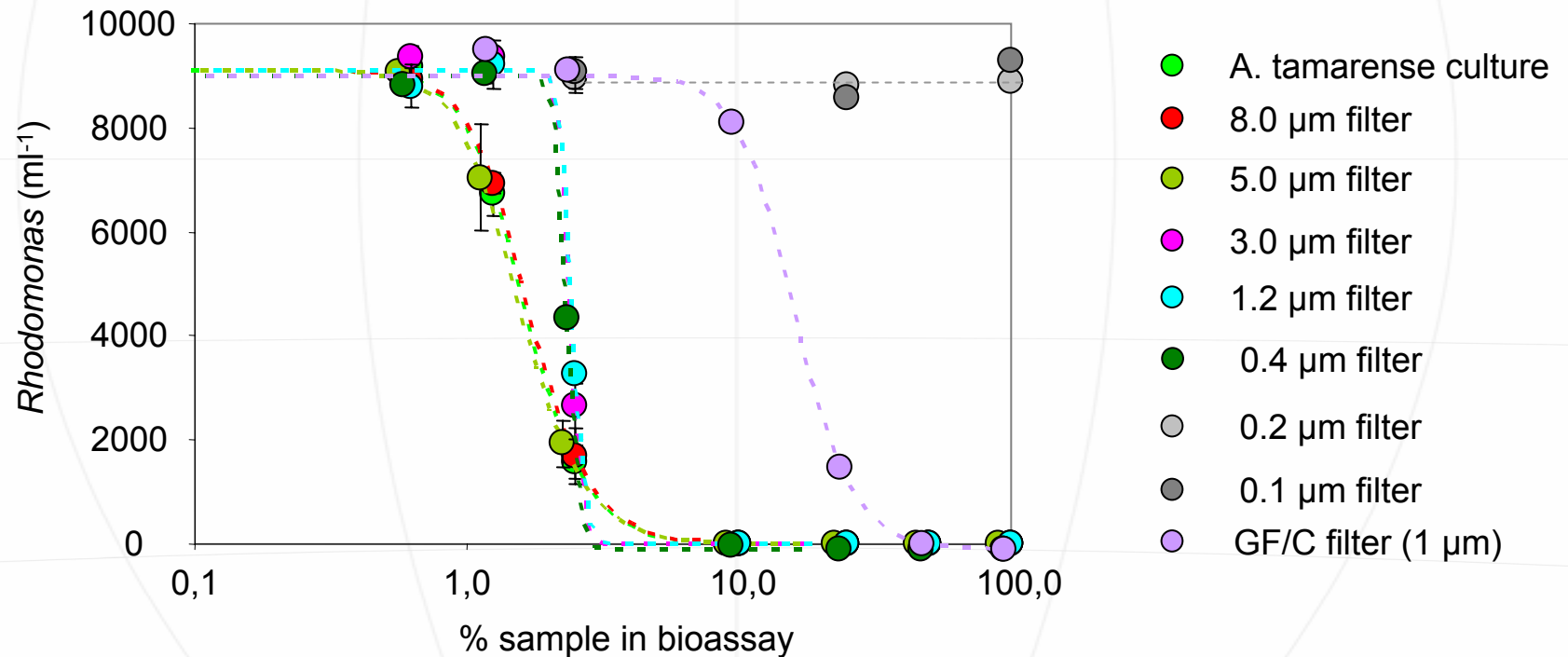


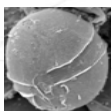
- t = 0
- t = 1d
- t = 4d
- t = 7d
- t = 12d
- t = 20d
- t = 49d



# Lytic Activity – Filterability

*Alexandrium tamarensis* supernatant – Lytic Effect on *Rhodomonas*

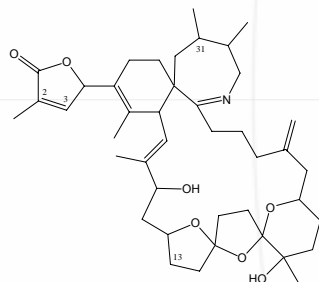




# Summary – *A. ostensfeldii* Allelochemistry

## Secondary Metabolite

Spirolides, marine toxins



?

## Ecological Function

?

Defense against Predators

Elimination of Competitors