



Re-Isolation of the azaspiracid producing dinoflagellate *Azadinium spinosum* from the Danish west coast

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Historic Background

Since 1995 new DSP-like poisoning events became public

MacMahon & Silke, 1996: Harmful Algae News, 14, 2

In 1998 Satake et al. identified azaspiracid-1 (AZA-1) as the causative compound in shellfish

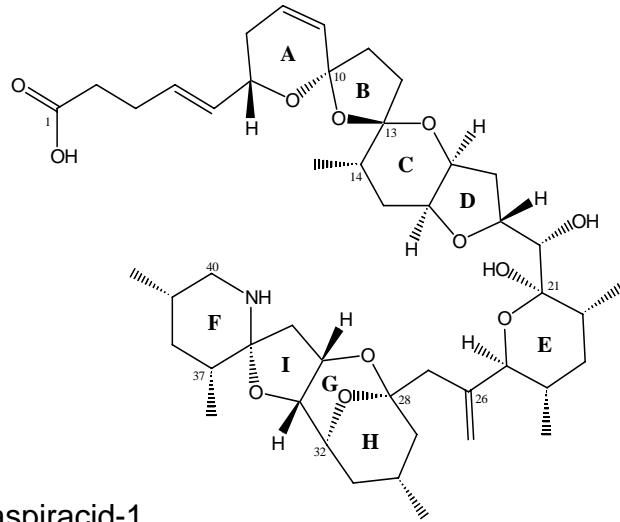
J. Am. Chem. Soc., 120, 9967-9968

In 2003 James et al. suggested the heterotroph dinoflagellate *Protoperidinium crassipes* as the AZP causing organism

Toxicon, 41, 277-283

To date more than 30 structural azaspiracid variants are known

Rehmann et al. (2008) Rapid Commun. Mass Spectrom., 22, 549-558



Azaspiracid-1



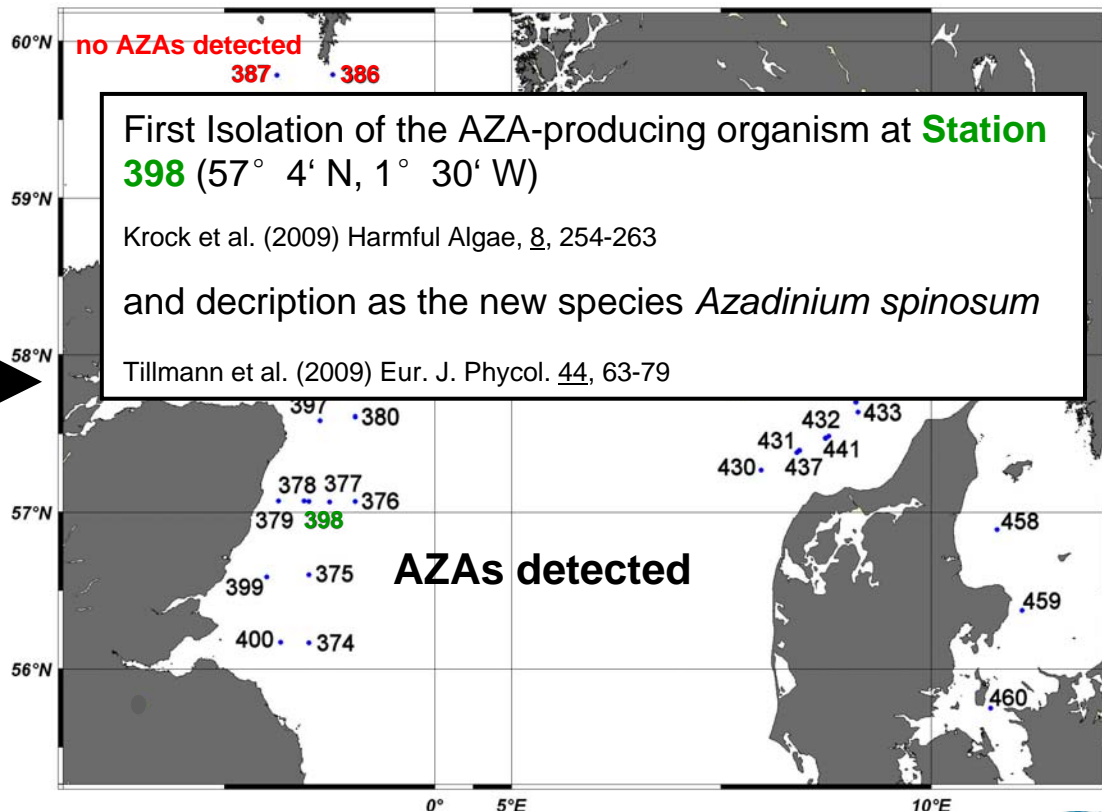
Historic Background

Moran et al. report that there was no correlation between the occurrence of *Protoperidinium* spp. in plankton and azaspiracids in shellfish in Irish waters over a four year period (2002 – 2006).

The authors exclude *Protoperidinium* as the source of azaspiracids

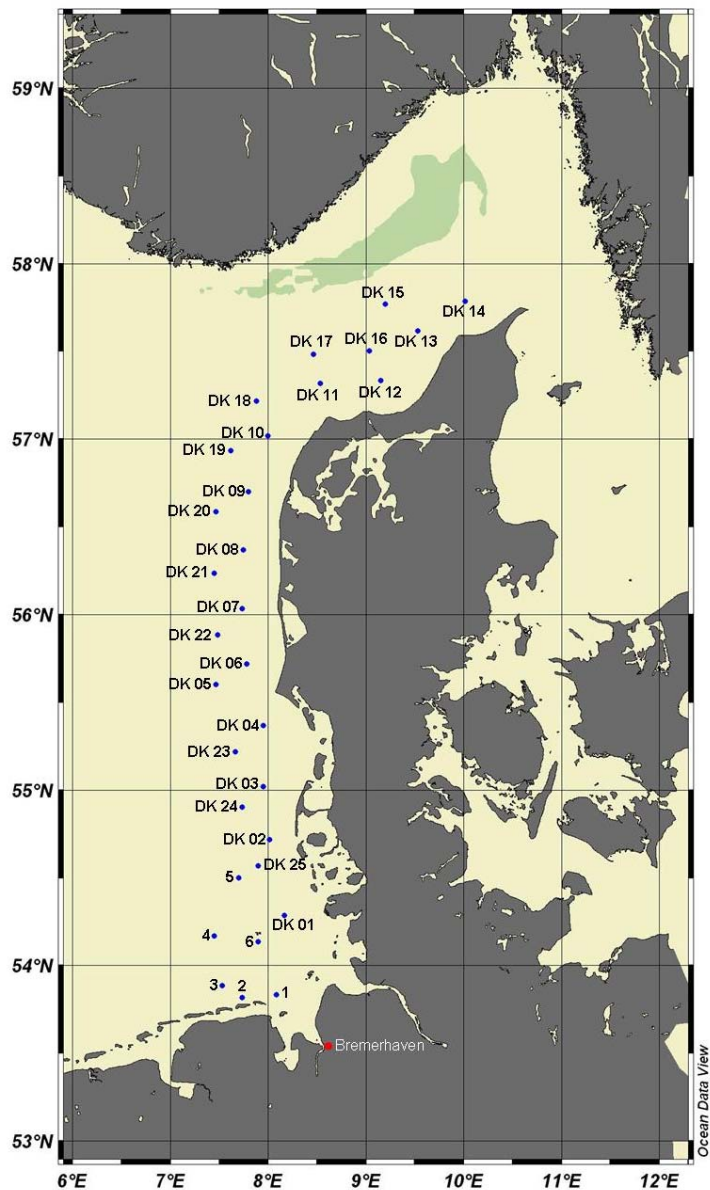


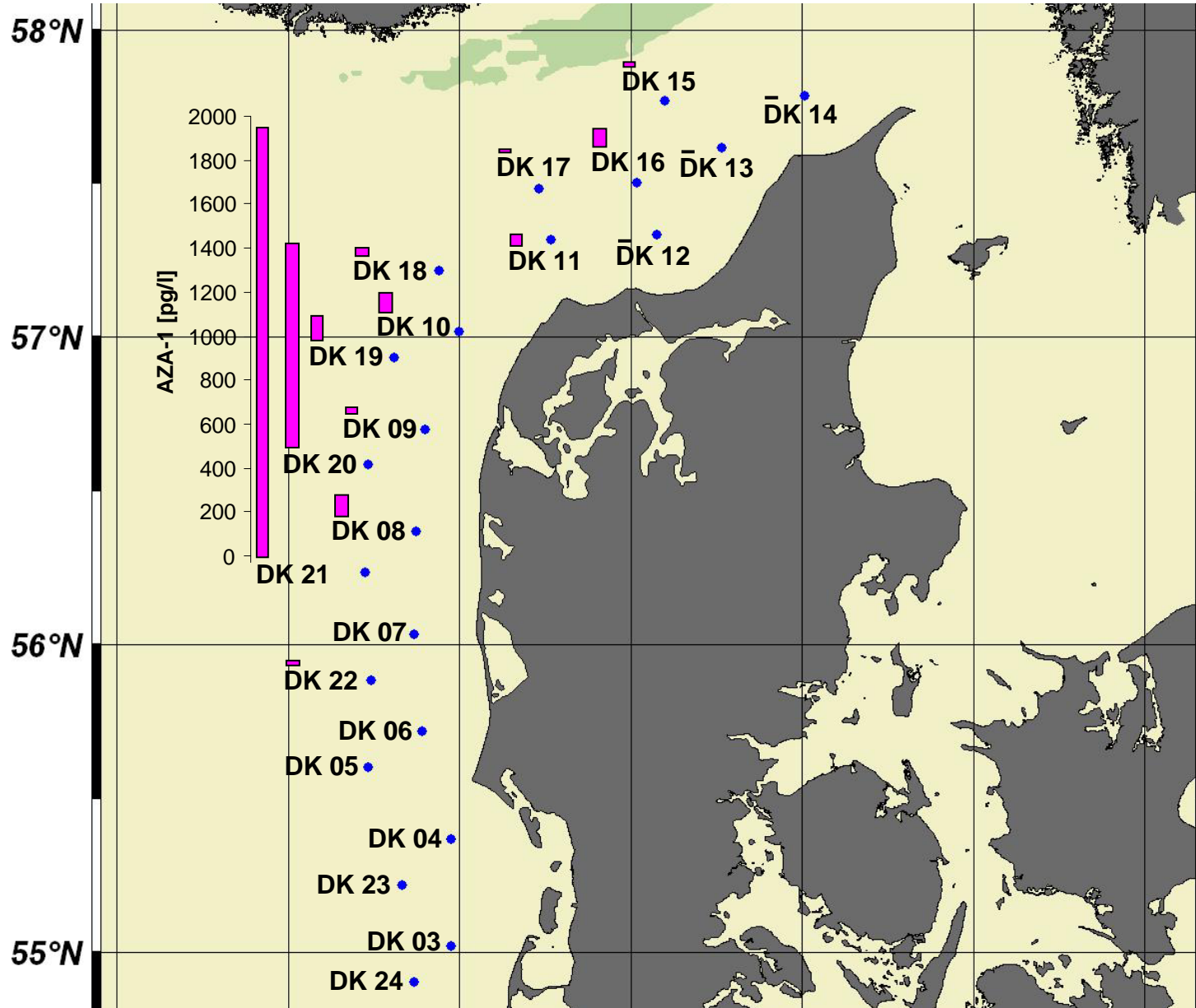
June/July 2007 +





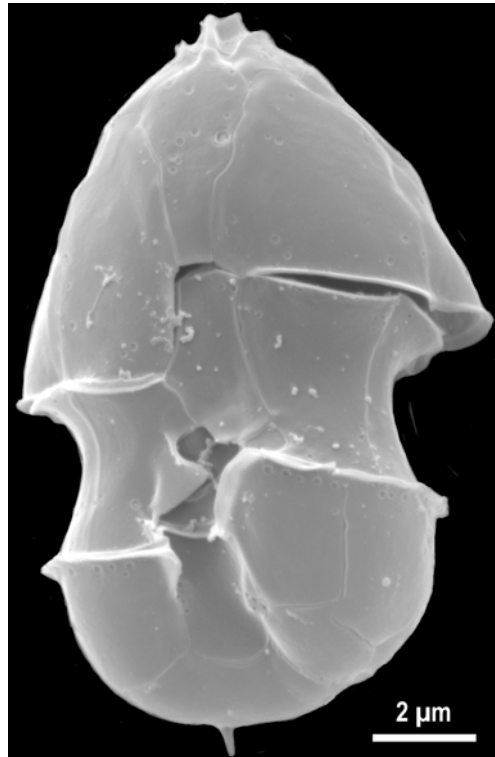
July 2008: FK Uthörn Research Cruise Bremerhaven - Skagen



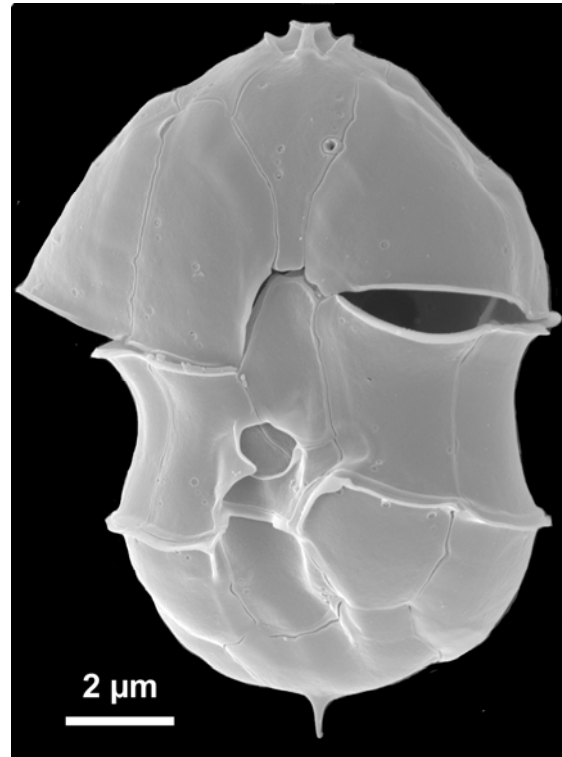




Second Isolation of *Azadinium spinosum* from Danish Waters
56° 15' N, 7° 28' E, (Station DK 21)



Azadinium spinosum
Strain 3D9
Scotland 2007

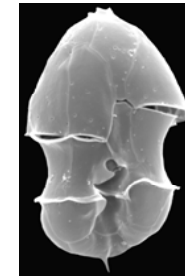


Azadinium spinosum
Strain UTHE2
Denmark 2008

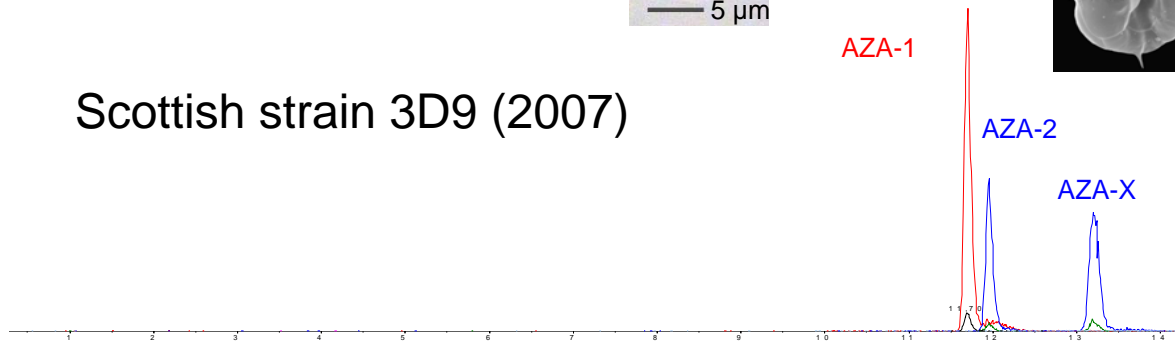


Second Isolation of *Azadinium spinosum* from Danish Waters 56° 15' N, 7° 28' E, (Station DK 21)

Two strains available:

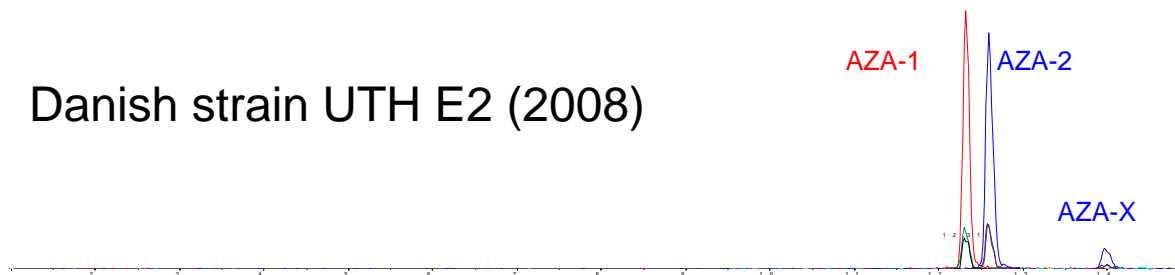


Scottish strain 3D9 (2007)



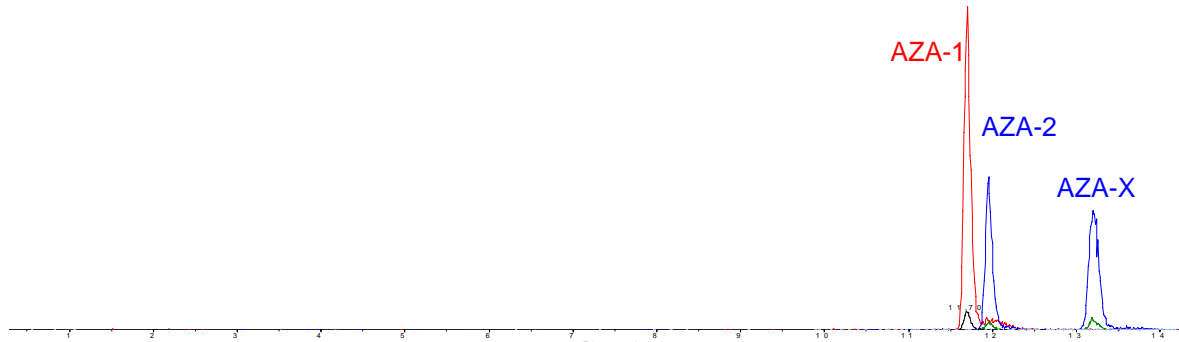
m/z 842>824
m/z 856>838

Danish strain UTH E2 (2008)

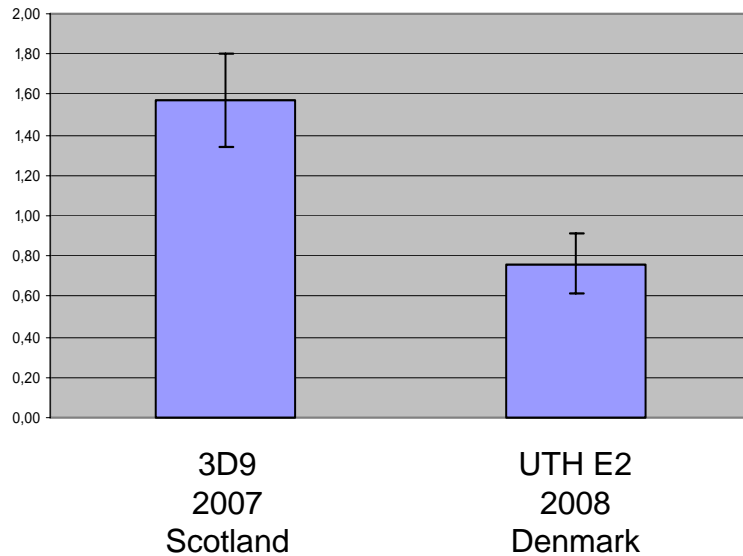




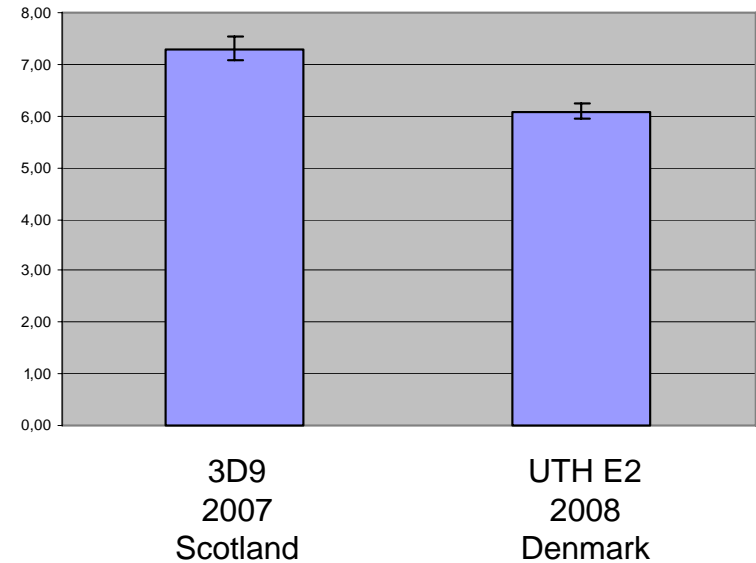
Toxin Profiles of 3D9 and UTH E2



Ratio AZA-1/AZA-2

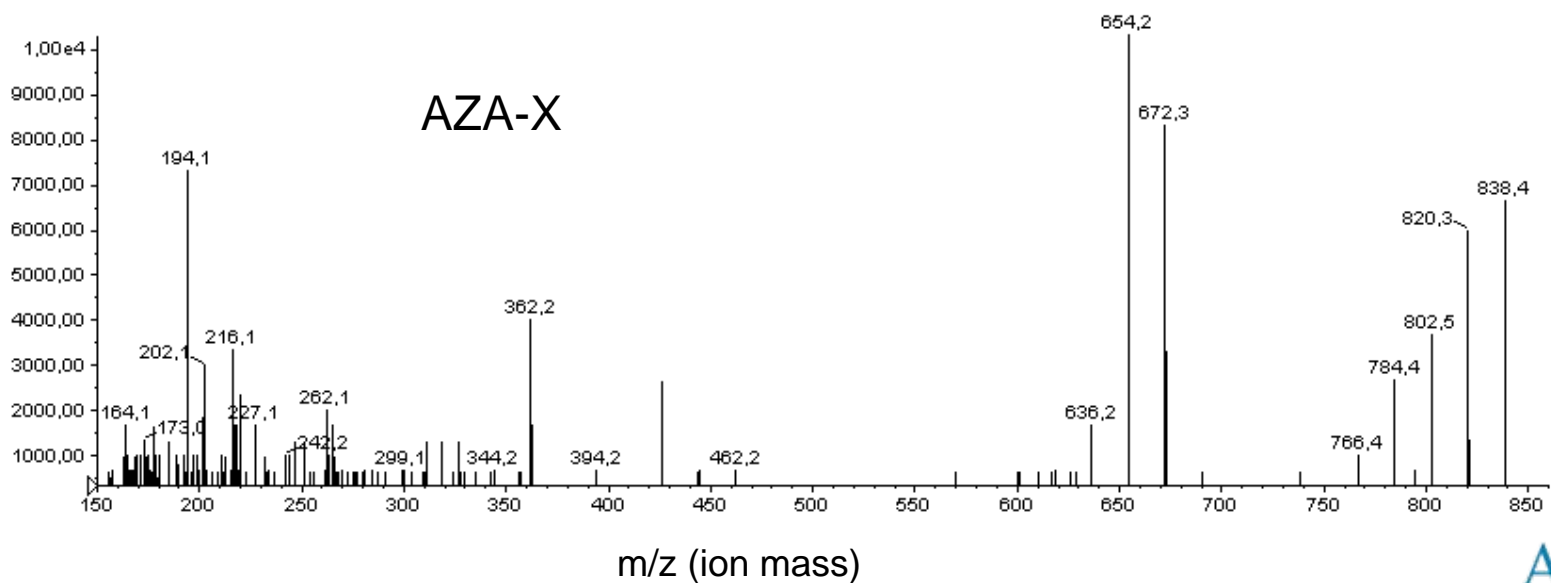
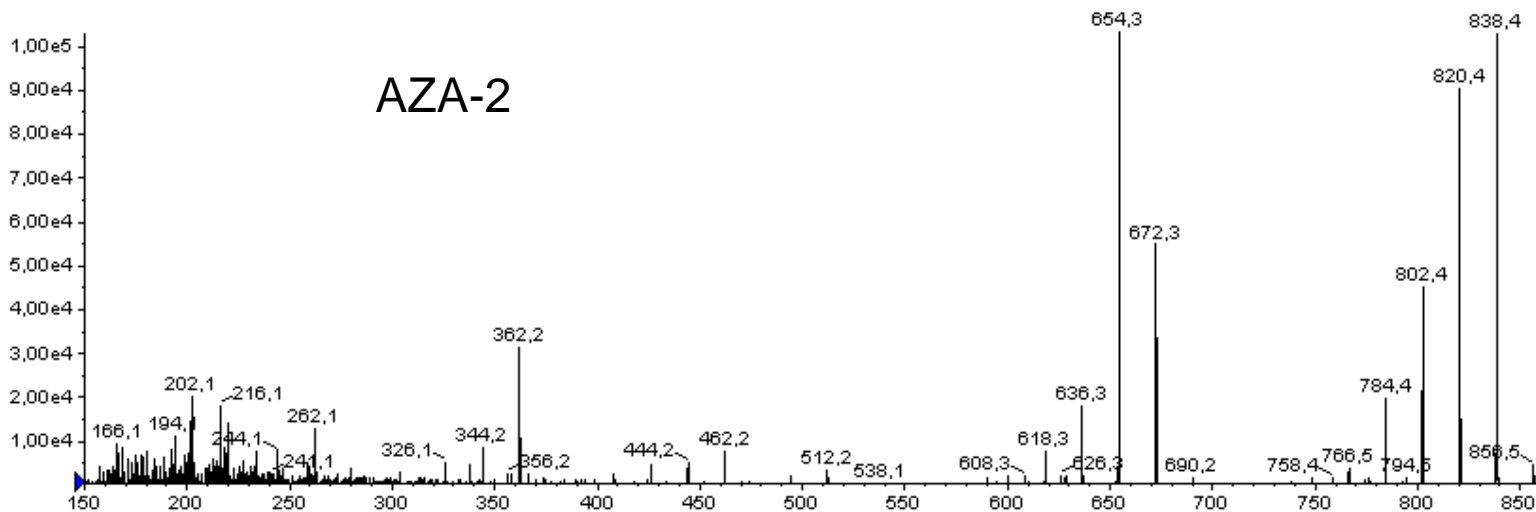


Ratio AZA-1/AZA-X





Comparison of the Product Ion Mass Spectra of AZA-2 and AZA-X





Azadinium – Light Microscopy



Azadinium spinosum



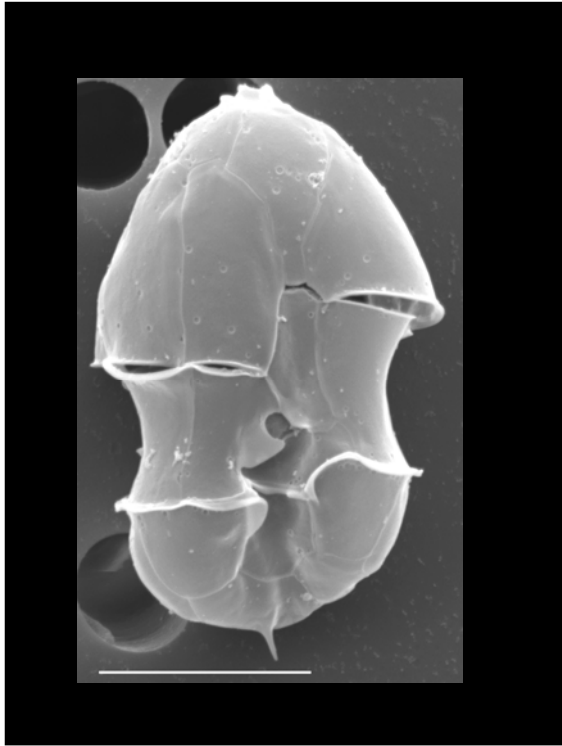
Azadinium sp. 2
Scotland
2007



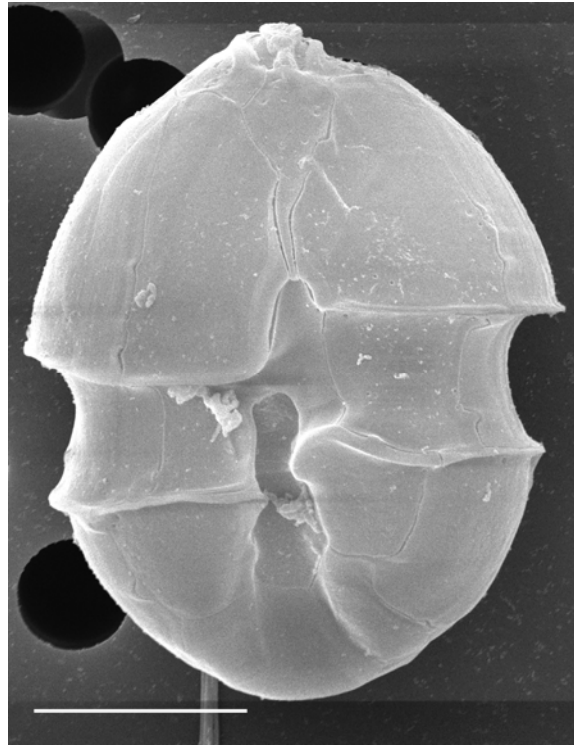
Azadinium sp. 3
Denmark
2008



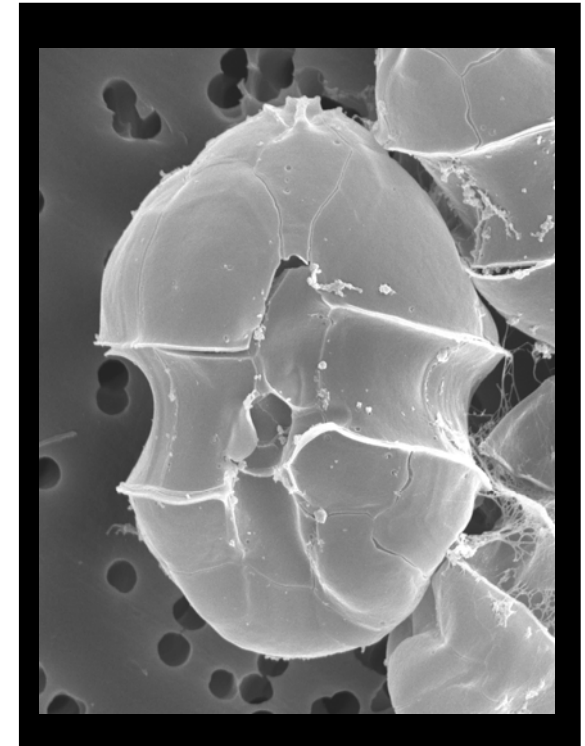
Azadinium – Scanning Electron Microscopy



Azadinium spinosum



Azadinium sp. 2
Scotland
2007



Azadinium sp. 3
Denmark
2008

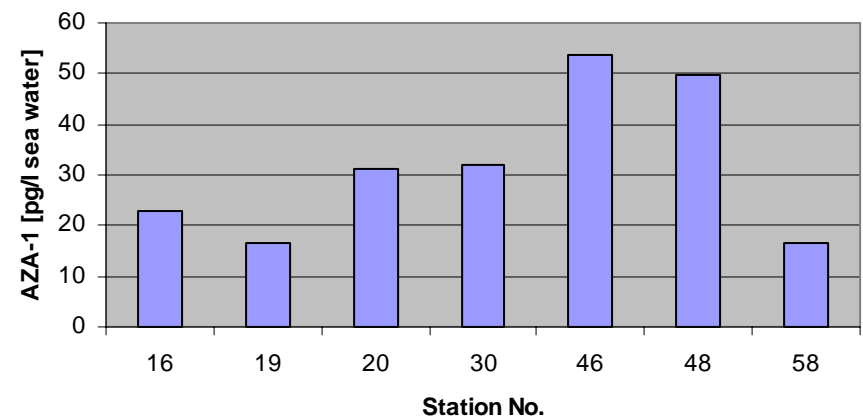
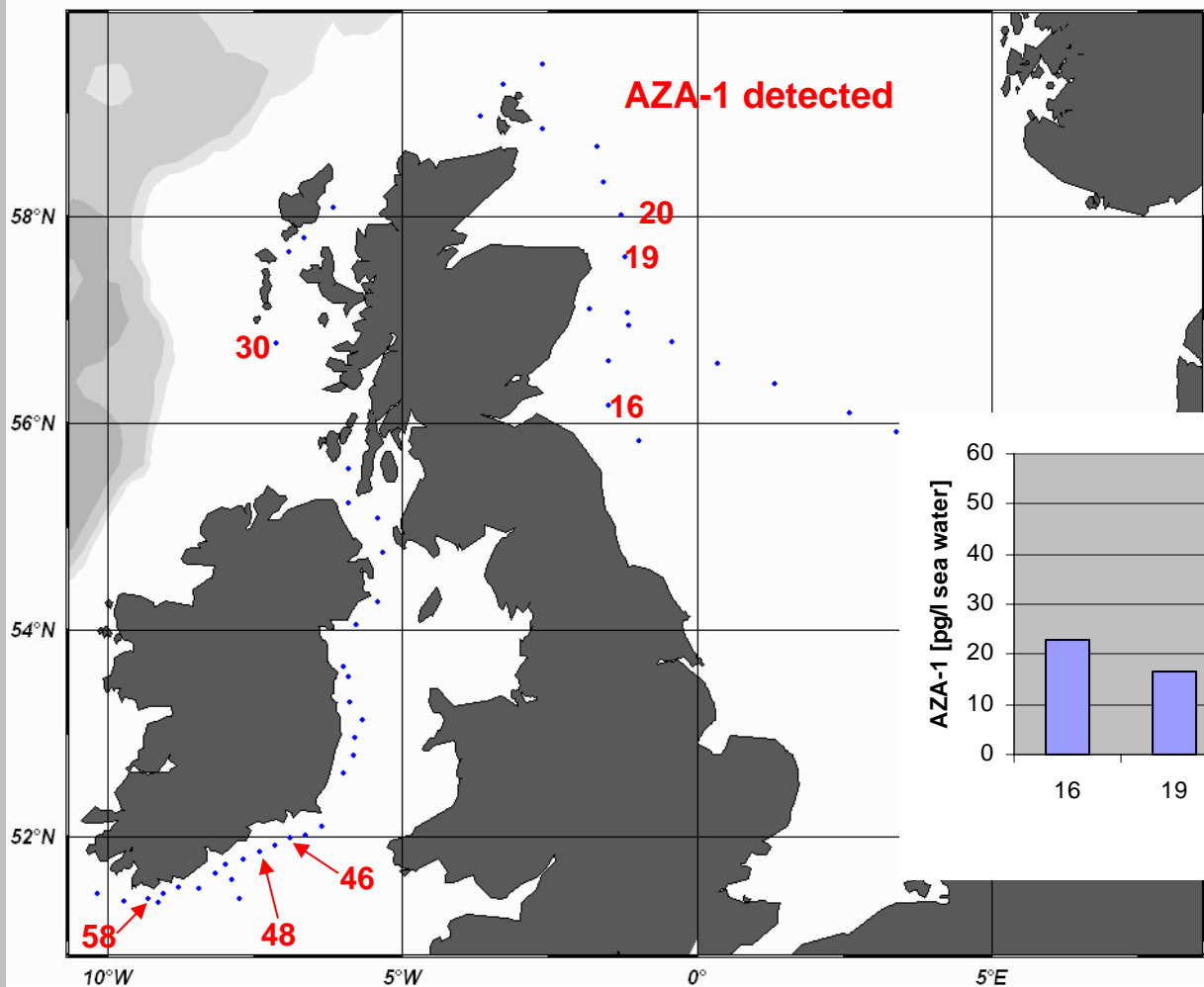


Azadinium – Species Comparison

strain	species	isolation	azaspiracid
3D9	<i>A. spinosum</i>	Scotland 2007	+
2E10	<i>Azadinium sp. 2</i>	Scotland 2007	-
UTH D4	<i>Azadinium sp. 3</i>	Denmark 2008	-
UTH C5	<i>Azadinium sp. 3</i>	Denmark 2008	-
UTH C8	<i>Azadinium sp. 3</i>	Denmark 2008	-
UTH E2	<i>A. spinosum</i>	Denmark 2008	+



April/May 2009: FS Heincke Research Cruise North Sea - Irish Sea - Celtic Sea





April/May 2009: FS Heincke Research Cruise
North Sea - Irish Sea - Celtic Sea



Live Samples HE 302 (Station 48)

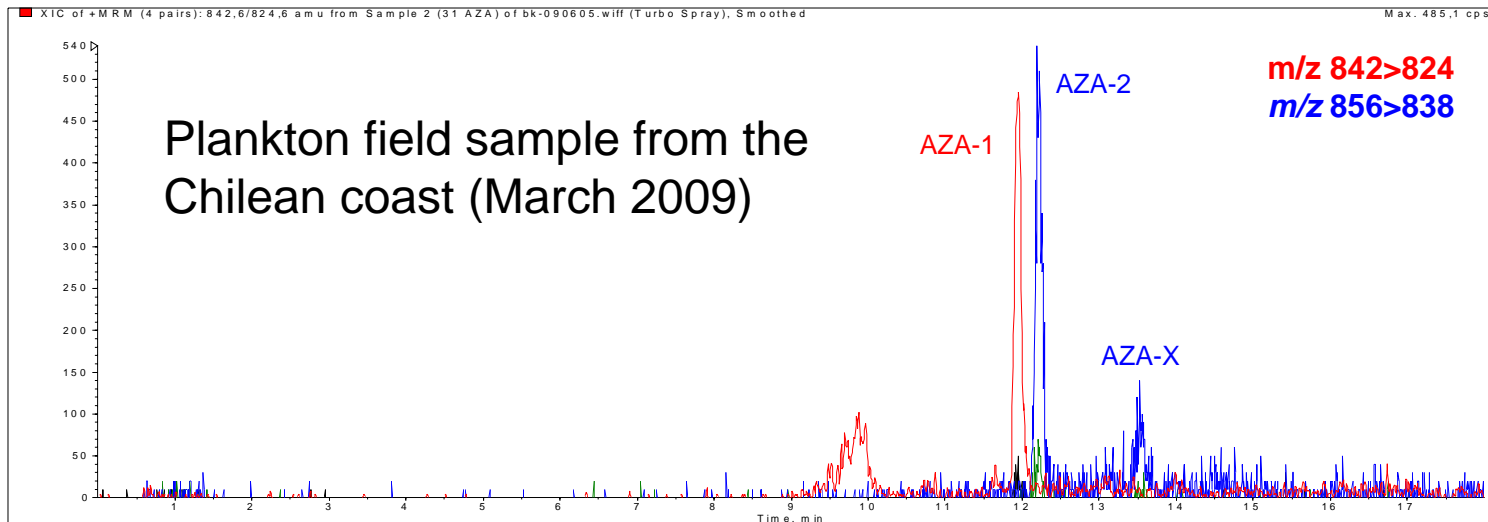
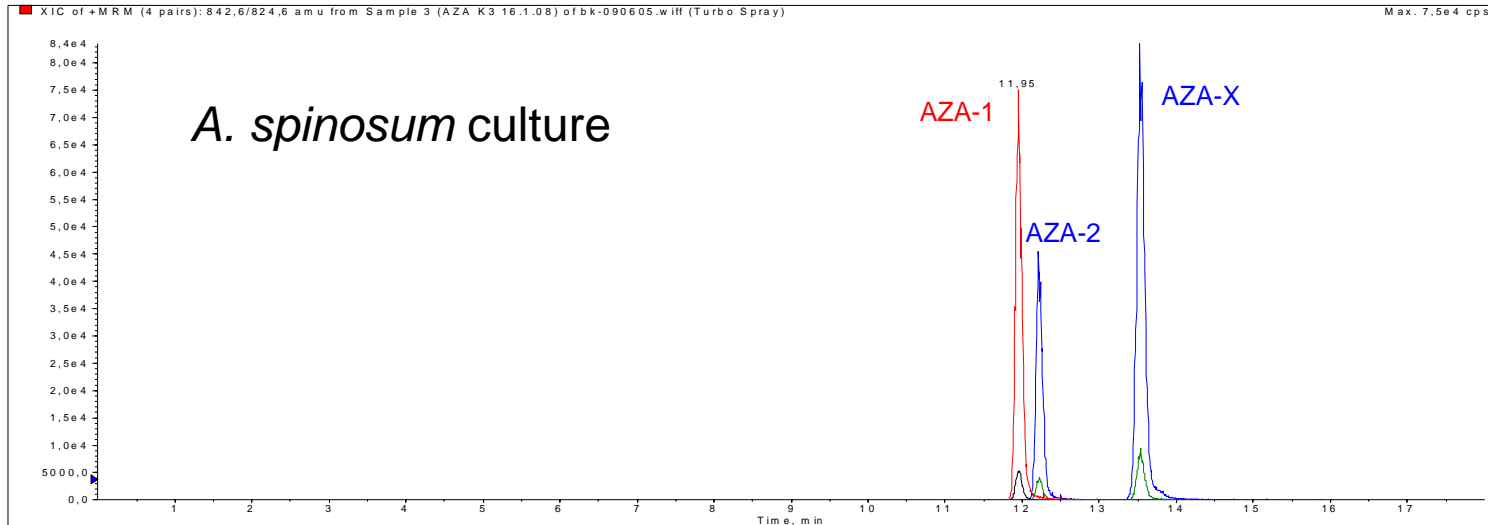
2 l sea water concentrated on 3 μm filters



Azadinium spinosum



Indication for AZAs in the South Pacific





Conclusion

1. To date *A. spinosum* seems to be the only source for azaspiracids (AZAs)
2. *A. spinosum* produces only AZA-1, AZA-2 and an isomeric form AZA-X
All other azaspiracids seem to be shellfish metabolites derived from these three compounds
3. Two other species of the genus *Azadinium* do not produce AZAs
4. *A. spinosum* is ubiquitous in the North East Atlantic
5. There is indication of the presence of *A. spinosum* in the South Pacific (Chile)



Thanks to...



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FK Uthörn Crew
FS Heincke Crew

...and for your attention