

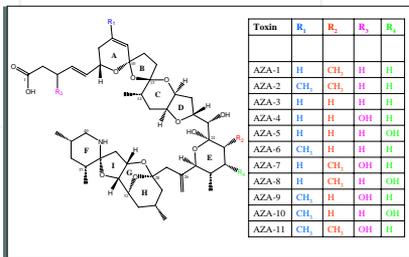
LC-MS/MS On Board – Isolation of an Unknown Toxin Producer from the North Sea



An API 4000 QTrap LC-MS/MS system on board the research vessel FS Poseidon

AZASPIRACIDS

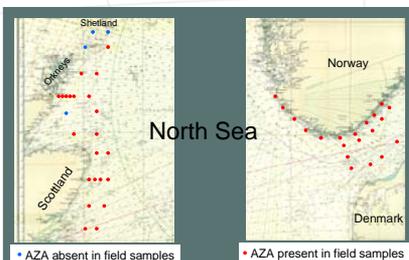
Azaspiracids are a group of lipophilic algal toxins initially associated with the diarrhetic shellfish poisoning (DSP) syndrome. The first azaspiracid poisoning (AZP) event occurred after 8 people in the Netherlands became ill in November 1995 after consumption of mussels from the Irish west coast [1]. Symptoms were nausea, vomiting, severe diarrhea and stomach cramps. In 1998, azaspiracid-1 (AZA-1) was structurally described from shellfish extracts as the compound responsible for AZP [2]. To date 11 AZA variants are known [3-5]. The producing organism was first attributed to the heterotrophic dinoflagellate *Prorocentrum crassipes* [5] but this has been subsequently disputed.



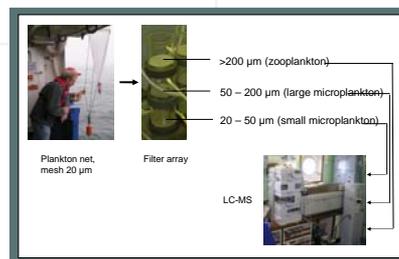
Azaspiracids: structural variants

Field samples

Azaspiracids were detected at most sampling stations in the North Sea. In 20 µm-mesh plankton net tows, highest amounts of AZA-1 were typically in the 50 - 200 µm size-fraction.



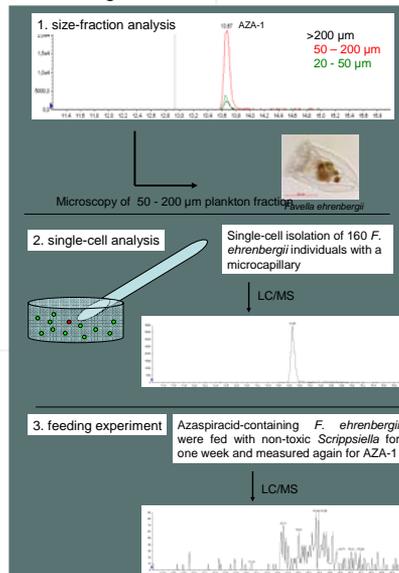
Occurrence of azaspiracids in the North Sea



Plankton fractionation and LC-MS analysis

Search for the AZA-producing organism

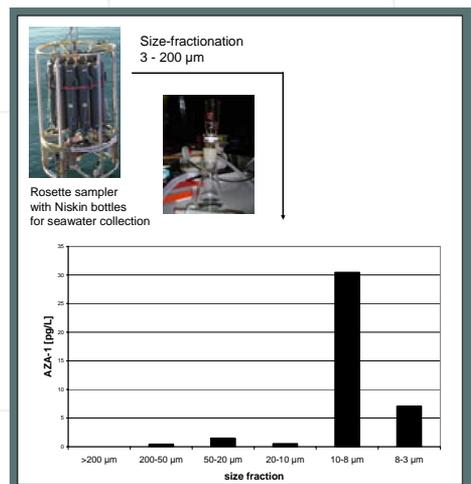
The 50 - 200 µm size-fraction contained mainly of the ciliate *Favella ehrenbergii*, which indeed contained AZA-1. However, after feeding *F. ehrenbergii* with the non-toxic dinoflagellate *Scrippsiella trochidea* for one week, no AZA-1 could be detected in these cells, which excludes *F. ehrenbergii* as the source of AZAs.



Analysis of net tow size-fractions and the organism that correlated best with the abundance of AZA-1

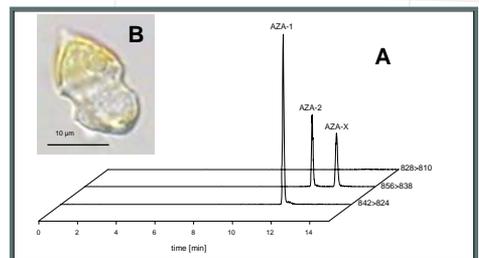
Direct water sampling and isolation of the AZA-producer

Seawater collected from Niskin bottle sampling at discrete depths and subsequently filter-fractionated revealed the highest amount of AZA-1 in the 8 - 10 µm size-fraction.



Abundance of AZA-1 in seawater size fractions

From this size-fraction, a small thecate phototrophic dinoflagellate was isolated by the serial dilution method. Once established in culture, this isolate proved to produce AZA-1, AZA-2 and an as yet undescribed isomer of AZA-2.



A: LC-MS/MS chromatogram of the AZA-producing isolate; B: Light microscopic photograph of the AZA-producing dinoflagellate

Precise taxonomic and genetic description of the AZA-producing dinoflagellate is currently underway.

References:

- [1] McMahon, T., HAN, 1996, 14, 2.
- [2] Satake, M., JACS, 1998, 120, 9967-9968.
- [3] Ofuji, K., Nat. Toxins, 1999, 7, 99-102.
- [4] Ofuji, K., Biosci, Biotechnol, Biochem, 2001, 65, 740-742.
- [5] James, K.J., Toxicon, 2003, 41, 277-283.