Bernd Krock[1], Urban Tillmann[1], Uwe John[1], Allan D. Cembella[1]

Isolation of the Azaspiracid-Producing Dinoflagellate *Azadinium spinosum* and Detection of Lipophilic Algal Toxins along the Danish North Sea Coast

On board the research vessel FK Uthörn

Introduction

Algal toxins pose a serious threat to human health via the accumulation in shellfish, which are increasingly cultured and harvested worldwide. Among these toxins are linear polyethers as okadaic acid, its derivatives and pectenotoxins, ladder shaped polyethers like yessotoxins, and cyclic imine toxins such as spirolides, gymnodimines, and azaspiracids. The aim of a research cruise performed in July 2008 was to survey the occurrence of these phycotoxins in the North Sea by liquid chromatography coupled to a triple quadrupole tandem mass spectrometer and subsequent isolation of the producing organisms.

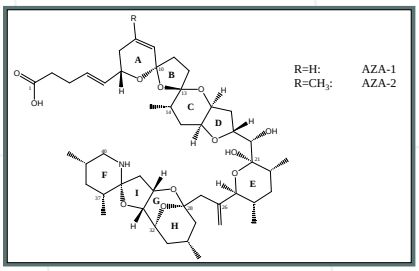

Fig. 1: Structures of azaspiracid-1 and -2

Azaspiracids (AZAs)

Azaspiracids are a group of lipophilic algal toxins associated with the diarrhetic shellfish poisoning (DSP) syndrome. The first azaspiracid poisoning (AZP) event was known after eight people in the Netherlands had become ill in November 1995 after consumption of mussels from the Irish west coast [1]. Symptoms of the affected persons were nausea, vomiting, severe diarrhea and stomach cramps. In 1998 Satake's group isolated and structurally elucidated azaspirazid-1 (AZA-1) as the causative compound in shellfish (Fig.1) [2]. In 2007 the producing organism was isolated for the first time from the North Sea [3] and described as the new species *Azadinium spinosum* [4].

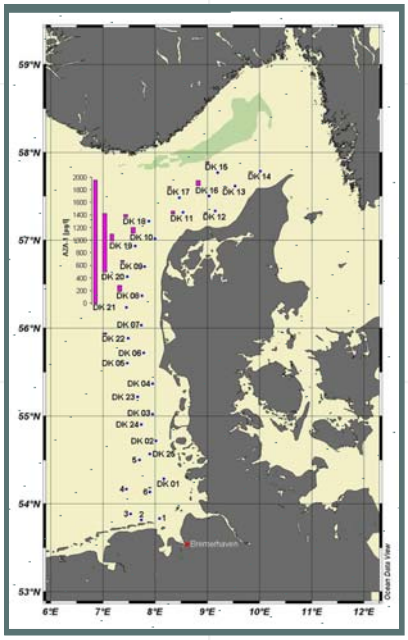

Fig. 2: Geographic stations of the 2008 FK Uthörn cruise with trespective AZA-1 concentrations

Isolation of *A. spinosum*

Analysis of Niskin bottle water samples revealed that highest abundances of AZA-1 were found in the 10-8 µm size fraction and AZAs were detected at most sampling stations in the North Sea north of 55°50' N latitude with the highest abundance at station DK 21 (56° 14.52' N, 007° 27.54' E) (Fig. 2). One clonal strain of *A. spinosum* provisionally designated as dinoflagellate isolate UTH E2 was established from a water sample collected from this station, which produces AZA-1 and AZA-2 in equal amounts (Fig.3).

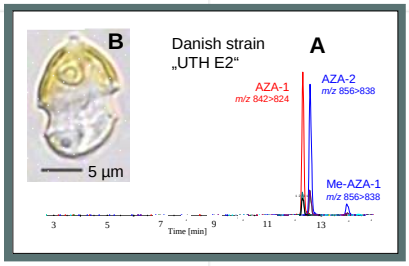

Fig. 3: A. spinosum A: toxin profile determined by LC-MS/MS B: light microscopy image

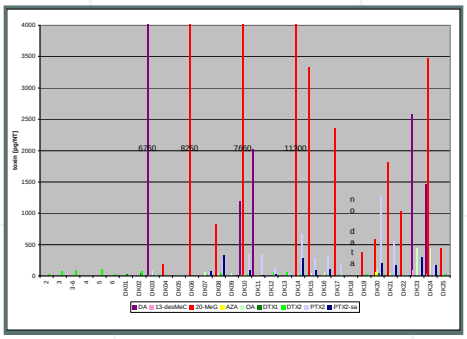

Fig. 4: abundances of domoic acid (DA), spirolides 13-desmethyl C and 20-methyl G, AZA-1, okadaic acid (OA), DTX1 and -2 and PTX2 and PTX2-seco acid in the 20-55 µm size fractions of plankton net tows.

Other lipophilic toxins

The phycotoxins detected on the cruise through the German Bight and along the Danish west coast, were 13-desmethyl spirolide C, 20-methyl spirolide G, AZA-1, dinophysistoxin-2 (DTX-2), pectenotoxin-2 (PTX-2) and domoic acid (DA). DTX-2 was detected at all stations and ranged from 0.3 to 630 pg/net tow with highest abundances in the > 200 µm size fractions. In contrast pectenotoxin-2 was only detected in northern Danish waters in the 55-20 µm fraction to a concentration of 1260 pg/net tow (Fig.4). Both toxins are known to be produced by the genus *Dinophysis*, but since there was no correlation between the occurrences of both toxins, it can be assumed that they were produced by different *Dinophysis* species. The most abundant toxin detected on this survey was 20 methyl spirolide G up to concentrations of 11300 pg/net tow. 13-desmethyl spirolide C was also detected, but at much lower levels (max. 88 pg/net tow). Both toxins correlate well geographically and are a strong indication for the presence of the marine dinoflagellate *Alexandrium ostenfeldii*.

References:

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[4] Tillmann, U., Eur. J. Phycol. 44, 63 - 79.

Alfred-Wegener-Institut
für Polar- und Meeresforschung
in der Helmholtz-Gemeinschaft

www.awi.de

1) Alfred Wegener Institute
Am Handelshafen 12
27570 Bremerhaven, Germany