THE FIRST DETECTION OF YESSOTOXIN FROM GONYAULAX SPINIFERA IN THE BENGUELA CURRENT UPWELLING SYSTEM

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REPUBLIC OF NAMIBIA
Ministry of Fisheries and Marine Resources
Presentation outline:

- Introduction
- Sampling methodology
- Results and discussion
- Current activities
Introduction:

Harmful algal blooms:

- Proliferation of phytoplankton up to millions of cells per liter.

- **Classifications (Hallegraeff 1995):**

  - Cause $O_2$ depletion in water column therefore kill indiscriminately. e.g. *Ceratium furca*
  
  - Non toxic to humans but causes chemical and mechanical damage to fish gills. e.g. *Chaetoceros convolutus*
  
  - Produces potent toxins causing gastrointestinal and neurological illness. E.g. PSP, DSP, ASP and NSP.
Sampling site:

- Sampling every fortnight
- Testing parameters: Temperature, dissolved oxygen, chlorophyll, phytoplankton ID, PO₄, SiO₄, NO₂ and NO₃
- Bloom conditions during 15-18th March 2011
- Coincided with H₂S in lagoon
- Daily sampling during bloom period for nutrients and environmental parameters
- Mussel and phytoplankton samples taken during this period

Satellite image: 15 March 2011
Dinoflagellate bloom:
Phytoplankton composition:

Phytoplankton species diversity from 15-18th March 2011

- Ceratium dens
- Ceratium furca
- Ceratium fusus
- Gonyaulax spinefera
- Gyrodinium sp.
- Noctiluca scintillans
- Prorocentrum cf triestinum
- Prorocentrum micans
- Protoperidinium pentagonum
Namaqua during the *Gonyaulax spinifera* bloom:

Temperature (°C)

Dissolved oxygen (ml l⁻¹)

Phosphate (µM)

Chlorophyll (µg l⁻¹)
Possible source of bloom:

1) Warm bottom water and stratification of the water column
   Cyst germination from sediment

2) Influx of seawater from outside the Bay area

GENUS cruise February 2011
High concentration of Gonyaulax spp

Anja Hansen, IOW
G spinifera identification

SEM photographs IOW
Yessotoxin (YTX):

- Disulfated polyether toxins
- Produced by *Lingulodinium polyedrum, Prorocentrum reticulatum* and *Gonyaulax spineafera*
- Previously categorised as DSP toxin
Yessotoxins: Global distribution

Presence of YTX in shellfish (●) and identification of YTXs in *P. reticulatum* (x)

Paz et al, 2008
Method used to measure YTX:

- LC-MS/MS
- Calibration with certified reference Standards of YTX
- Analogues detected by hydrolysis
Results: YTXs in mussel samples

- First detection of YTX in shellfish in the Benguela
- Oysters tested positive for DSP during bloom period
- Low abundance of carboxy YTX unusual as it is generally the second most abundant analogue
YTX in phytoplankton field samples:

- Total YTX was produced at 156.0 pg cell\(^{-1}\)
- homoYTX being produced at 96.0 pg cell\(^{-1}\)
- Three fold of that of a comparable spp. from the Adriatic sea
- First detection of 45-OH YTX as main YTX analogue
- First detection of YTX producing *G. spinifera* in the Benguela region
Current activities:

- **Laboratory experiments with G spinifera**
  - 01 March 2012 bloom found adjacent to Aquapark 1 (22° 56’.448” S, 14° 29’.352” E)
  - Species composition: *P tristenum, P micans, C furca* and *G spinifera*
  - 11 monoclonal *G spinifera* cultures
  - (½) F/2 media with filtered seawater
  - 17°C with a 14:10h L:D cycle

- **Phylogenetic work on cultures:**
  - DNA extraction
  - Amplification (PCR) of large and small sub-units of ribosome (LSU and SSU rDNA)
Molecular phylogeny
SSU
Molecular phylogeny LSU

Genetically similar to the Adriatic strain
Macronutrient experiments:

- Determining the effects of PO$_4$ and NO$_3$ on growth, toxin production (including analogues)
- Mimic environmental conditions since cultures are grown in excessively nutrient rich media

**Table 2:** Nutrient experiment with various concentrations

<table>
<thead>
<tr>
<th>Experimental ID</th>
<th>Sample type</th>
<th>Phosphate ($\mu$M)</th>
<th>Nitrate ($\mu$M)</th>
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<tr>
<td>1</td>
<td>F/2 media*</td>
<td>Control</td>
<td>20.347.24</td>
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<tr>
<td>2</td>
<td>Nut. test 1</td>
<td>Low PO$_4$</td>
<td>0.5</td>
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<tr>
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<td>Nut. test 2</td>
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<td>5</td>
<td>Nut. test 4</td>
<td>Low NO$_3$</td>
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<tr>
<td>6</td>
<td>Nut. test 5</td>
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<tr>
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<td>Nut. test 11</td>
<td>Mix 5</td>
<td>43.0</td>
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</table>
YTX in field and culture samples:
Outputs:

- *Gonyaulax spinifera* from the Benguela upwelling system: Toxin production and phylogeny
- *Gonyaulax spinifera* from the Benguela upwelling system: Effects of nutrients on growth and toxin production

Focus for the future:
- Feeding experiments with oysters and mussels to determine toxicity thresholds
- Generation of molecular probes of culture strains
- Using probes for FISH on field samples from 2011 and 2012 to determine species diversity
- Expand toxin testing along Namibian coastline (i.e. GENUS II)
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