Toxicon 56 (2010) 1487–1496

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Production of diarrhetic shellfish poisoning toxins and pectenotoxins at depths within and below the euphotic zone

Elie Fux [a,*], Sonsoles Gonzalez-Gil [b], Michel Lunven [c], Patrick Gentien [c], Philipp Hess [a,1]

[a] Biotoxin Chemistry – Marine Institute, Rinville, Galway, Ireland
[b] Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Spain
[c] Ifremer, Centre de Brest, BP 70, 29280 Plouzane, France

ARTICLE INFO

Article history:
Received 11 July 2010
Received in revised form 13 September 2010
Accepted 22 September 2010
Available online 1 October 2010

Keywords:
Marine toxins
Dinophysis acuta
LC–MS
Passive sampling
SPATT
Okadaic acid
Dinophysistoxin-2
Pectenotoxin-2

ABSTRACT

During a 10 day survey in the CelticSea near the Irish South-West coast (July 2007), *Dinophysis acuta* was observed in large numbers. The deployment of a profiler allowed for the identification of a *D. acuta* thin layer that reached 1910 cells/L. The aim of the study was to investigate if the bloom that occurred in low light environment was viable, dividing, actively producing toxins and if the toxin profile changed over a short term period. Several large concentrates of phytoplankton samples were obtained over a 14 h period, from evening to morning, by pumping *Dinophysis* from specific depths. In addition, *D. acuta* was collected in complete darkness at 81 m depth by concentrating 120 L of water. The cells were extracted and their toxin profiles were established by liquid chromatography – mass spectrometry (LC–MS). Passive samplers were deployed in a nearby location for 6 days at 30, 50, 70 and 110 m depth, and the toxin profiles were determined by LC–MS as above. The toxin profiles obtained in phytoplankton samples and in the SPATT were compared and correlated well. Sample concentrates and SPATT results suggested that toxic *D. acuta* occurred and produced similar toxin profiles at all water depths, including below the euphotic zone.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Dinoflagellate species of the genus *Dinophysis* produce marine toxins which can accumulate in shellfish due to their filter feeding nature. The consumption of contaminated shellfish can result in a variety of symptoms including the diarrhetic shellfish poisoning (DSP) caused by the toxins okadaic acid (OA) and dinophysistoxins (DTX). Therefore, the occurrences of *Dinophysis* along coastal areas have been responsible for the closure of shellfish production areas worldwide resulting in economic losses.

The main toxin producing species within the *Dinophysis* genus include *Dinophysis acuminata*, *Dinophysis acuta*, *Dinophysis fortii* and *Dinophysis norvegica*. Analysis of hand-picked cells or of concentrates of these species by liquid chromatography coupled to mass spectrometry (LC–MS) have shown that these strains produce toxins of the pectenotoxin (PTX) and the okadaic acid group (Lee et al., 1989; MacKenzie et al., 2002; Miles et al., 2004a, 2004b; Hackett et al., 2009). The amount of toxins produced and their relative abundance can vary depending on the strain and also on the geographical origin of the phytoplankton. For example, *D. acuminata* from Japan produces OA as well as DTX1 (Lee et al., 1989; Suzuki et al., 2009; Kamiyama and Suzuki, 2009) and *D. acuminata* from France was shown to produce OA but no DTX1 was detected (Marcaillou et al., 2001, 2005). The occurrence of a high density of *D. acuminata* without mussel contamination was also reported in Japan (Hoshiai et al., 1997). The differences in the nature of

* Corresponding author. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, 1 Iberville Dr, Dauphin Island, AL 36528, USA, Tel.: +1 251 690 2342.
E-mail address: elie.fux@marine.ie (E. Fux).
[1] Current address: Ifremer, Centre de Nantes, BP 21115, 44311 Nantes CEDEX 03, France.

0041-0101/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2010.09.007

the toxins produced and the occurrence of harmful phytoplankton without apparent toxicity in shellfish demonstrate that toxicity of a population of phytoplankton can be difficult to establish by relying solely on cell identification. We recently investigated the use of the passive sampler solid-phase adsorption toxin tracking (SPATT) developed by MacKenzie et al. (2004), for the determination of toxin profiles in a culture of *Prorocentrum lima* (Fux et al., 2008) as well as in the field, for the comparison of toxin profiles in shellfish and in passive samplers (Fux et al., 2009).

In the present study we report the presence and the production of toxins by *D. acuta* cells that were collected within and below the euphotic zone. The experiment was conducted in the CelticSea as part of a 10-day survey for the study of harmful algae blooms in thin layers. The production of toxins over time was studied in cells collected in the thin layer over a 14 h period and analysed by LC–MS/MS. The presence of toxins in above and below the euphotic zone was also monitored at various depths using SPATTs that were deployed at the beginning of the survey. This approach enabled to obtain a time integrated concentration of the extracellular toxins at depths as low as 110 m. In addition, we report the presence of toxins in one sample of *D. acuta* collected in complete darkness at more than 80 m depth.

2. Material and methods

2.1. Solvents and reagents

Acetonitrile was purchased as pestiscan grade (Labscan Ltd., Dublin, Ireland) and dichloromethane (DCM), hydrochloric acid (HCl, 37%) and sodium hydroxide (NaOH) were obtained as analytical grade (Merck, Darmstadt Germany). Formic acid and ammonium formate were obtained from Sigma–Aldrich, UK (F-0507 and F-2004, respectively). Water was obtained from a reverse osmosis purification system (Millipore, Bedford, USA). Standards of OA and PTX2 were purchased as certified calibration solutions from the National Research Council (Halifax, Canada). OA-D8 and PTX11 reference solutions were generously donated by Dr. Michael Quilliam and by Dr Chris Miles, respectively.

2.2. The Pelagic profiler

At each station, measurements and sampling in the water column were performed with the high resolution IFREMER profiler (IPSAP) which integrates a SBE25 CTD probe (Sea-Bird Electronics, Washington, USA), a CILAS (Orléans, FRANCE) particle size analyser (PSA) and a video system. This profiler allows real time visualization of ca. 60 parameters every 2 s. It provides guidance for sampling *Dinophysis* since this species is associated with specific conditions (salinity discontinuities, aggregates, specific shape of the size distribution histogram) as reported in Gentien et al. (1995).

2.2.1. Deployment and sampling strategy

At each station, the IPSAP profiler was lowered at a speed of 0.3 m/s. Real time data acquisition allowed an accurate description of the physical and chemical characteristics of the water column and the detection of the maximum chlorophyll layer and the location of layers possibly containing *Dinophysis*.

During the up-cast, finer-scale measurements were performed within the water layers and several stops were made at selected depths to collect samples from structures of interest (fluorescence maximum, pycnocline, maximum particle load, zooplankton abundance). Sampling within these layers were carried out using a 40 mm diameter hose which was attached to the profiler, close to the sensors, allowing a peristaltic pumping of water at a rate of 30 L/min. Peristaltic pumping with a wide bore pipe ensured that minimal damage was caused to the organisms and allowed collection of cells from the exact position where they had been detected. The phytoplankton composition of the collected samples was systematically determined on-board using an inverted microscope.

2.2.2. The particle size analyser (PSA) and the CTD probe

Based on diffraction pattern analysis, the PSA measures the total volume of particles present in an 8 mL free flow cell and their size distribution over 30 size classes from 0.7 to 400 μm (Gentien et al., 1995). This method enables the quantification of phytoplankton populations, organic and inorganic particles and organic matter aggregates. This PSA is associated with the SBE25 probe to allow real time data acquisition of standard parameters such as depth, temperature, salinity, chlorophyll-like fluorescence and photosynthetically active radiation (PAR).

2.3. Collection of D. acuta cells

2.3.1. Pumping at maximum chlorophyll layer – 14 h study

Concentrated filtrates of *D. acuta* were obtained on the 25th of July 2007.

Samples were collected off the South-West of Ireland (Lat. 08°57.03 W and Lon. 51°27.26 N) (Fig. 1). Water samples were obtained every 3 h at the maximum chlorophyll layer located just above the thermocline using the profiler's peristaltic pump and filtered over a 200 μm and a 20 μm mesh. The depth of the layer varied from 22 to 34 m depending on the time of the day. The cells collected on the 20 μm mesh were scooped in a jug, concentrated in 150 mL and the homogenous solution divided into 3 × 50 mL centrifuge tubes. A small fraction (2 mL) was used to determine the viability of the cells (as described in Section 2.11). One 45–50 mL sample was preserved in lugol for cell counts and another 50 mL sample was used for toxin profile determination.

2.3.2. Deep D. acuta sample

One sample was collected just above the sediments (81 m) on the 27 July by concentrating 138 L into 50 mL (Lat. 7°34.91 W; Lon. 51° 28.02 N) (Fig. 1).

2.4. SPATT design and handling

The SPATT discs were prepared as previously reported (Fux et al., 2008). A circular frame was used as resin holder instead of the resin filled sachets described by MacKenzie et al. (2004).

Download English Version:

https://daneshyari.com/en/article/2065064

Download Persian Version:

https://daneshyari.com/article/2065064

Daneshyari.com