Toxicon 108 (2015) 202–209

Contents lists available at ScienceDirect

Toxicon

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

Assessment of emerging biotoxins (pinnatoxin G and spirolides) at Europe's first marine reserve: Lough Hyne

Moira McCarthy [a, e, *], Vaishali Bane [b], María García-Altares [c], Frank N.A.M. van Pelt [d, e], Ambrose Furey [b], John O'Halloran [a, e]

[a] School of Biological, Earth and Environmental Sciences, University College Cork, The Distillery Fields, North Mall, Cork, Ireland
[b] Mass Spectrometry Research Centre (MSRC) Incorporating the PROTEOBIO and Team Elucidate Research Groups, Department of Chemistry, Cork Institute of Technology (CIT), Bishopstown, Cork, Ireland
[c] IRTA, Carretera de Poble Nou, km 5.5, 43540 Sant Carles de la Ràpita, Spain
[d] Department of Pharmacology and Therapeutics, University College Cork, Western Gateway Building, Western Road, Cork, Ireland
[e] Environmental Research Institute, University College Cork, Lee Road, Cork, Ireland

ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form
14 September 2015
Accepted 13 October 2015
Available online 23 October 2015

Keywords:
SPATT
Biotoxin
Pinnatoxin
Spirolide
HABs
LC-MS

ABSTRACT

Active and passive sampling methods were employed over a four-month period, at a site off the South-West coast of Ireland, to characterise the occurrence of cyclic imines in the water column. The marine toxins 13-desmethyl-SPXC, 20-methyl SPXG toxins and pinnatoxin G were detected using active sampling from Diaion HP-20 resin. Seven water depths were sampled to determine stratification of the toxins in the water column using Solid Phase Adsorption and Toxin Tracking (SPATT). Both 13-desmethyl-SPXC and pinnatoxin G were detected using two different resin types; Diaion HP-20 and Amberlite XAD761. HP-20 proved more effective at accumulating the toxins, with a higher percentage of positive samples and a higher ratio of toxin adsorbed relative to XAD761. No temporal variation in toxin-quantities was detected, indicating that there was no change in density of causative algal species in the water column. Pinnatoxin G was detected more frequently from surface to 30 m depth, with a similar pattern observed for 13-desmethyl-SPXC occurrence using XAD761. No difference in the occurrence of 13-desmethyl-SPXC was observed between depths using HP-20 resin. This is the first reported incidence of pinnatoxin G in Irish waters and highlights cyclic imines as emerging toxins in European waters.

1. Introduction

A small proportion of Harmful Algal Blooms (HABs) can produce toxins that accumulate in the tissues of other organisms, particularly filter-feeding bivalves. A number of these filter-feeders are important aquaculture species for human consumption worldwide. These toxins have harmful impacts on human health and cause the closure of shellfish farms, particularly during the summer months, leading to negative socio-economic effects (Anderson et al., 2000; Hoagland et al., 2002). Rising global ocean temperatures, increased occurrence of extreme weather events (such as El Niño), as well as growing coastal eutrophication, have all been linked to an increase in the incidence of HABs worldwide (Anderson et al., 2002; James et al., 2010). The growing geographical spread of these harmful species has been attributed to ballast waters transporting encysted algae to new environments and similarly, spread of algae by practises in aquaculture (Anderson et al., 2002; Masó and Garcés, 2006; Smayda, 2007; van Dolah, 2000). Our greater ability to monitor and identify these toxins at very low levels using analytical methods could also be an explanation for their increased detection (Draisci et al., 2000; Furey et al., 2005).

Cyclic Imines (CIs) are a family of marine biotoxins which includes spirolides (SPXs) and Gymnodimines (GYMs) produced by algal species from the genus *Alexandrium* and *Karenia*. Based on the chemical structure spirolide, spiro-prorocentrimine, pinnatoxin, pteriatoxin and gymnodimine are grouped as cyclic imines. The producer of pinnatoxin F and G in Australia, New Zealand and Japan has been identified as the dinoflagellate *Vulcanodinium rugosum*,

* Corresponding author. AltraTech Ltd., Cavanagh Pharmacy Building, University College Cork, Cork, Ireland.
E-mail addresses: mc_moira@yahoo.co.uk (M. McCarthy), vaishali.p.bane@mycit.ie (V. Bane), maria.garciaaltares.perez@gmail.com (M. García-Altares), f.vanpelt@ucc.ie (F.N.A.M. van Pelt), Ambrose.Furey@cit.ie (A. Furey), j.ohalloran@ucc.ie (J. O'Halloran).

http://dx.doi.org/10.1016/j.toxicon.2015.10.007
0041-0101/

previously found in Mediterranean field samples (Rhodes et al., 2011) (Table A1). These toxins can accumulate in shellfish tissues (Hu et al., 2001; McCarron et al., 2012). Spirolides were first identified in Canadian Shellfish and since then, have spread to many countries world-wide (Botana, 2014) (Table A2). Pinnatoxins, which are structurally similar, have only recently been identified in European waters in Norwegian blue mussels and seawater (Rundberget et al., 2011), since that time they have been detected in France and Spain (Garcia-Altares et al., 2014; Hess et al., 2013). Many CIs have been discovered to be toxic to mice by intraperitoneal injection in doses ranging from 12.7 to 57 μg/kg body weight for pinnatoxin E, F and G; and concentrations of 6.9–99 μg/kg body weight for spirolides, A, B, C, 13-desmethyl-C and 20-methyl spirolide G (Munday et al., 2012a, 2012b). However, when administered with food CIs have proven less toxic, in some cases by an order of magnitude. An exception to this was recorded with pinnatoxin F also exhibiting a low LD_{50} when the toxin was given with food indicating a high oral bioavailability (Munday et al., 2012b). Based on the toxicological studies to date, it has been concluded that some CIs have a neurotoxic effect and can bind and block acetylcholine receptors in central and peripheral nervous systems (EFSA, 2010). At present there are no regulatory limits for CIs in shellfish tissues in Europe, or other regions of the world and there are no long-term studies examining the chronic impacts of these toxins to determine a tolerable daily intake.

Passive monitoring using Solid Phase Adsorbent and Toxin Tracking (SPATT) and active sampling methods for the detection of marine biotoxins directly from water have been developed (MacKenzie et al., 2004; Rundberget et al., 2007). Both methods rely on using an adsorptive resin which can accumulate lipophilic toxins which 'leak' from the algal cell and persist in the water (MacKenzie et al., 2003). SPATT has been successfully used in a number of studies as an early-warning system to detect lipophilic marine biotoxins prior to the occurrence of a bloom event (Rodríguez et al., 2011; Turrell et al., 2007). Active toxin sampling involves the pumping of water through a series of filtration devices and through a cartridge containing the resin (Diaion HP-20) based on a design by Rundberget et al. (2007). In that study large quantities of okadaic acid (OA) and dinophysis toxin-2 (DTX-2) were successfully accumulated. Use of an adsorptive resin for the accumulation of biotoxins has some advantages over the use of shellfish tissues for the direct detection and characterisation of toxins present in the marine environment. The adsorption of the biotoxin is direct and there is no biotransformation of the toxins, such as fatty acid esterification found in shellfish (O'Driscoll et al., 2011; Vale et al., 1999). Lack of biotransformation coupled with relatively 'clean' sample matrices simplifies the extraction and analysis of toxins accumulated using this method (MacKenzie, 2010). For the additional assessment of human health implications of algal biotoxins accumulated through SPATT, analysis of the metabolites produced by shellfish species that accumulate these toxins is also valuable, as some of these metabolites can be harmful to human consumers of toxic shellfish.

A comprehensive profile of the phytoplankton assemblage present in the water column at Lough Hyne Marine Reserve from January 2008 to June 2009 detected a number of toxin-producing algal species (Jessopp et al., 2011), thus it was chosen as the study site for this research. Passive and active sampling methods have been successfully applied to profile the Diarrhetic Shellfish Poisoning (DSP) toxins present, both spatially and temporally at this site (McCarthy et al., 2014), highlighting the use of these methods in profiling and monitoring phytoplankton distribution in the water column. In the current study, samples were analysed to determine the presence of cyclic imines at Lough Hyne, as these toxins are becoming more prevalent in European waters. In addition the distribution of these toxins was characterised over a four-month period at the study site.

2. Materials and methods

2.1. Construction and deployment of the active toxin sampler

The active toxin sampler was based on the design of Rundberget et al. (2007). The sampler was deployed for 7 consecutive days at Lough Hyne Marine Reserve, Cork, Ireland (51°29′ 58″N 9°17′ 49″W), from August 24th – August 31st, 2010 and was operated continuously over the period, apart from 30 min on the 25th August, 2010 when the 50 μm bag filter was replaced to ensure no clogging occurred. Water flow was 6.67 l min^{-1} and the seawater pump was submerged at 1 m below the surface.

2.2. Construction and deployment of SPATT bags

SPATT bags were based on the design of MacKenzie et al. (2004), but modified slightly to include a Velcro re-sealable opening which allowed removal of the resin and the bags to be recycled. The 95 μm polyester mesh was purchased from John Staniar and Co., Whitefield, Manchester, UK. Bag proportions were 100 mm × 100 mm and a loop was sewed on one top corner to enable attachment to the submerged mooring line using zip ties. Two types of resin were used in this study. The first, Diaion HP-20 (Supelco, 13607) has a broad application base, it is a polyaromatic adsorbent resin for hydrophobic compounds such as biomolecules and antibiotics. The second, Amberlite ® XAD761 (Supelco, 10356) an adsorbent resin useful for the purification of pharmaceuticals, the removal of proteins, organic impurities and high MW colourants. The resins were activated as per the manufacturer's instructions, by soaking in methanol and rinsed using deionised water, 5 g dry weight (8.8 g wet weight) of HP-20; and 5 g dry weight (6.1 g wet weight) of activated XAD761were added to each SPATT bag. The bags were placed in ziplock plastic bags at 4 °C and kept damp until use as per Rundberget et al. (2009). SPATT bags were deployed for two week time intervals from May–August 2010. The bags were kept in airtight ziplock bags for transportation back to the laboratory and were stored at −20 °C until extraction.

2.3. Field site location

Lough Hyne Marine Reserve, Co. Cork, Ireland was chosen as the study location for this investigation. Three sites were chosen within the Lough, two sites of 20 m depth, the North Basin (NB) (51°50′32″N 9°30′15″W) and the South Basin (SB) (51°50′01″N 9°29′94″W) and one 50 m deep site in the Western Trough (WT) (51°50′08″N 09°30′42″W). The SPATT bags were attached at the surface, 5 m, 10 m and from then on at 10 m intervals from the surface until the sea-bed and replaced every two weeks. The active sampler was submerged in the South Basin (51°49′88″N 9°29′86″W).

In addition to deploying bags, vertical phytoplankton hauls were also taken with a net every two weeks. Identification of the phytoplankton was performed to determine the causative algal species. This was done for a subset of samples from August 2010, where a significant increase in some phycotoxin quantities was detected in the passive filters.

2.4. Solvents and reagents

Chemicals used for liquid chromatography coupled to mass spectroscopy (LC-MS) (HPLC Grade acetonitrile; ammonium acetate; trifluoroacetic acid; HPLC Grade Water), were purchased from